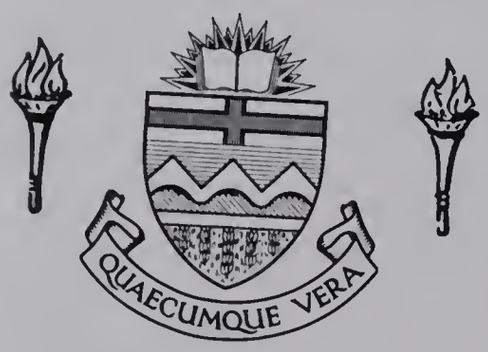


UNIVERSITY OF ALBERTA

INTEGUMENTARY GLAND CELLS OF EIGHT NORTH PACIFIC ASTEROIDS.

by

JON ANTHONY BENSON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN

PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

SEPTEMBER 1967

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Integumentary Gland Cells of Eight North Pacific Asteroids" by Jon Anthony Benson in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The aboral surfaces of eight asteroid species from the North West Pacific coast were examined with special reference to the structure and general histochemical properties of the glandular tissue. The animals used in this study were: Dermasterias imbricata; Luidia foliolata; Hippasteria spinosa; Mediaster aequalis; Pteraster tesselatus; Henricia leviuscula; Pycnopodia helianthoides; Leptasterias hexactis. In the cuticle which covers the epidermis, cytoplasmic protuberances extend from the cytoplasm of the cell to the environment. Some evidence of secretion from the tip of the protuberances has been observed when using the electron microscope. All gland cells were found to contain distinct secretory packets. When these packets were released to the environment it was either through a distinct secretory pore or by the apparent rupture of the gland cell wall. In all animals except Pteraster tesselatus, gland cells which had no apparent opening to the exterior were observed. The previous classifications of asteroid gland cells as mucus and muriform were suggested to be the products of paraffin embedding techniques. Epon 812 was shown to be a superior embedding compound for showing structural detail in glandular tissue. The major chemical components of the gland cells on the aboral surface of the asteroids studied appeared to be either acid mucopolysaccharide or a P.A.S. positive compound. In most cases this latter compound is thought to be a neutral mucopolysaccharide.

In four species, gland cells have been observed which apparently secrete a protein, and the possibility of gland cells which secrete lipid compounds was suggested in some animals. Without exception, the gland cells which did not open to the environment did not respond to any histochemical test or any routine stain which would distinguish them from the rest of the epidermis.

ACKNOWLEDGEMENTS

I would like to express my deep appreciation to my supervisor, Dr. D. M. Ross, for his help and consideration throughout this project. I would also like to give my sincere thanks to Dr. G. O. Mackie and to Dr. T. K. Shnitka for their help and encouragement. My appreciation to Dr. A. R. Fontaine who introduced me to this project, and to Dr. R. L. Fernald for the use of the facilities at the Friday Harbor Laboratories, University of Washington.

I would also like to express my thanks to members of the University of Alberta technical staff whose assistance was invaluable in this project: Mr. Ron Seward, Mrs. Violet Scott, Mr. G. Menges, and Mrs. L. Mitchell.

Thanks are also due to Mr. D. E. Goyette, without whom SCUBA collecting would have been much more difficult; and to Mr. R. Forchuk and Mr. B. Gillespie for assisting in the preparation of the manuscript.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

INDEX OF COMPARATIVE DATA ON THE ASTEROID GLAND CELLS

1. INTRODUCTION

The histology of the asteroid aboral surface is not well known. A paper published by Cuénot in 1887 was one of the first histological surveys in which a number of species were examined and compared. Cuénot worked with 18 species from the Red Sea, the Mediterranean Sea and the Atlantic Ocean. References to the fairly recent volume of the Echinodermata in Hyman's treatise, The Invertebrates (Vol. IV, 1955), and to the Physiology of the Echinodermata (Boolootian, ed., 1966) show that only scattered studies on isolated genera have appeared during the subsequent three-quarters of a century. It would appear that comparative histological studies dealing with the surface tissues of the asteroids are long overdue.

The Pacific Coast of Western North America is rich in asteroid species and provided excellent opportunities for embarking on comparative studies. Some of these Pacific species have been investigated in connection with problems confined to a single specie and were not exclusively histological in nature. Henricia sanguinolenta (Hayashi, 1935) and Pteraster tesselatus (Rodenhouse and Guberlet, 1946) have been studied in detail but with regard to the general morphology rather than any specific cellular or chemical region. Dermasterias imbricata is the only asteroid that has been studied until now with respect to the chemical components of the aboral gland cells (Ward, 1965). The remainder of the aster-

oids dealt with in this work have not been studied with respect to either structural or chemical composition of their aboral gland cells.

Asteroids in general produce a mucus secretion which covers their aboral surface. This process of secretion becomes more prominent when the animal is irritated either physically or chemically, and as such, appears to be a defensive substance. It would appear that the mucus contains some irritating substance which appears to produce a variety of responses in other marine animals. This mucus is thought to be the reason that no encrusting organisims have been observed attached to the surface of asteroids. In the case of Pycnopodia helianthoides a saponin-like toxin has been extracted (Rio. et al, 1963) but its cellular location has not been discovered. In some animals, e.g. Henricia leviuscula, the mucus is also thought to have a food trapping function in conjunction with its suggested ciliary feeding.

Very little is known about either the structural or chemical properties of the asteroid surface gland cells. In 1887, Cuénot used a classification of the asteroid gland cells based on their apparent structure when observed in microscopic sections. During this study the classification used by Cuénot was examined, and as the work developed, it became obvious that there were major problems in attempting to relate Cuénot's description of gland forms to the gland cells observed

in the Pacific coast asteroids. The primary task proved to be a comparison between the results obtained from paraffin embedding and embedding in modern synthetic resinswhich have recently become available. As a result, it will be suggested that paraffin embedding produces artifacts in glandular tissue, and as such should not be used in a system of classification. Some preliminary recommendations for the formation of a new system of classification are suggested.

The gland cells of eight species of asteroids were studied with respect to both their structural and chemical composition. Different types of gland cells were observed and tentatively classified within each species. In some instances general theories are proposed with regard to the function of specific gland types.

New areas of active secretion other than gland cells have been suggested by the discovery of cytoplasmic protrusions through the cuticle on the surface of apparently all epidermal cells.

When the gland cells can be identified both structurally and chemically, and the complete secretory capability of the aboral surface is understood, a more precise understanding of the environmental role of the mucus will be possible.

II. MATERIALS AND METHODS

1. Species

The species of asteroids used in this study are tabulated below: (following the description of Fisher 1911 - 30)

Order Phanerozonia

Dermasterias imbricata (Grube)

Luidia foliolata Grube

Hippasteria spinosa Verrill

Mediaster aequalis Stimpson

Order Spinulosa

Pteraster tesselatus Ives

Henricia leviuscula (Stimpson)

Order Forcipulata

Pycnopodia helianthoides (Brandt)

Leptasterias hexactis (Stimpson)

2. Introduction to Animals and Environments

The above mentioned are common animals on the North West Coast of North America. The collecting area was located in the waters of Puget Sound and the Strait of Juan de Fuca within the square encompassing 20° 00′ to 123° 40′ longitude, by 48° 10′ to 48° 50′ latitude. This area includes Victoria and the south coast of Vancouver Island, the Saanich Peninsula, the San Juan Isl.,

Port Angeles area, and the north half of the Washington coast that borders on Puget Sound.

SCUBA diving was used whenever possible to collect the animals in order to keep damage and irritation to a minimum. When this collection method was not feasible, the animals were obtained by dredging from the fifty-three foot converted trawler M. V. HYDAH. In both cases the collected animals were kept in fresh sea water aquaria in the marine laboratories at Friday Harbor, Washington, until examination.

Dermasterias imbricata, the leather star, is a subtidal species which is endemic to the Pacific North West Coast of North America, and is the only endemic phanerozonid species apt to be taken in shore collecting (Hyman, 1955). It is found sparingly on rocky bottom from Monterey Bay to Alaska and was reported in Ricketts and Calvin (1953)to be delicately purple with red markings. It has been reported that D. imbricata feeds on injured holothurians and crustacea (Feder & Christensen, in Boolootian, 1967) and may be a scavenger. This animal is one of the two asteroids which will cause the seimming response in the sea-anemone Stomphia coccinia by physical contact with the asteroid's mucus (Yentsch and Pierce, 1955). This animal was collected for this study by SCUBA diving in the waters around the southern tip of Vancouver Island and around San Juan Island.

Luidia foliolata lacks the stiff, star-like shape of many phanerozonians and instead has long, flexible arms and a

relatively small disk (Hyman, 1955). This animal is characteristic of the littoral zone from California to Alaska (Hyman, 1955). The specimens used in this study were collected from a depth of 100 feet in Satellite Channel at the Northern tip of the Saanich Penninsula by SCUBA diving. The animal's coloration is a dull gray with a dark stripe down the centre of each of the rays. L. foliolata is an active predator of ophiuroids and will cause them to move away as it approaches.

Hippasteria spinosa has an aboral surface which is bright red in color. This is a species which occurs on the Pacific Coast from California to Alaska but which is limited to the deeper water of the littoral zone (Hyman, 1955). As a rule it is found on muddy bottoms. No feeding information is available for H. spinosa in its natural environment, but in a laboratory tank this asteroid will eat only shellfish which have had their shells damaged. This suggests that the animal may be a scavenger or specializes in eating a type of food which was not available in captivity. H. spinosa was obtained by dredging at a depth of approximately 130 feet in Satellite Channel. This animal will, along with Dermasterias imbricata, produce the swimming response in Stomphia coccinia by contact with the mucus produced by the asteroid (Sund, 1958).

Mediaster aequalis is a brilliant red asteroid, which is prominently flattened in a dorso-ventral plane. It occurs on the Pacific coast from California to Alaska (Hyman, 1955). No feeding habits have been reported for this animal. M. aequalis

was collected in Satellite Channel at depth at 60 to 100 feet by SCUBA diving and also by dredging in various channels of the same depth around the San Juan Islands. The mucus of this animal produces no known response in other animals.

Pteraster tesselatus, the cushion star, presents a very characteristic appearance in that it is a plump, five rayed asteroid which exhibits a large pore in the middle of its aboral surface. This animal is found from Puget Sound to the Bearing Sea (Hyman, 1955). Its natural color ranges from an off-white to a deep gold, although it is most commonly found as a dull yellow. The food of Pteraster tesselatus consists of sponges and hydroids (Rodenhouse & Guberlet, 1946). In the undisturbed state, this animal has a thin film of viscous mucus covering its surface. However in response to irritation, large amounts of mucus are rapidly liberated. This secretion has been reported as being toxic to a variety of other animals including crabs (Rodenhouse and Guberlet, 1946). In my experience, however, kelp crabs will appraoch P. tesselatus and feed on the mucus secretion.

This animal was collected in 15 to 40 feet of water by SCUBA diving at Saxe Point in Victoria and from the same depth during various dredging operations around the San Juan Islands.

Henricia leviuscula the Blood Star, may have a circumboreal range, as does Henricia sanguinolenta (Hyman,1955). Henricia leviuscula has as its main characteristics

the marked roundness and rigidity of its five arms, a dull red color, and small surface spines arranged in a regular pattern which could be missed by a casual observer. The habitat of this asteroid is usually substratum of rock, and not of mud. It has been reported to be a filter feeder but some doubt exists on this point. When an undisturbed animal was touched, no discernible mucus was felt, but on handling the animal a slight secretion was observed.

H. leviuscula was collected by SCUBA diving and occurred from 10 to 40 feet below the surface at Saxe Point, Victoria; the breakwater, Victoria; Albert Head, Vancouver Island; and also in various dredging operations among the shallower channels between the San Juan Islands. The mucus or contact ofthis animal produces no response in other animals except a response to the physical stimulus.

Pycnopodia helianthoides, the 20-rayed star, is a large asteroid with from 15 to 23 soft rays whose entire aboral surface is covered with groups of branched dermal branchiae, bushy paxillae, and stalked pedicellariae (Hyman, 1955) (Fig. 51). This species occurs from Southern California to the Aleutian Islands on rocky bottoms and is a conspicuous member of the fauna of Puget Sound (Hyman, 1955). The coloration of P. helianthoides varies from a general orange-red to a green-purple with an orange-red margin. The animal is a voracious feeder which preys on gastropods, crustacea, echinoderms, algae,

sponges, and clams (Feder & Christensen, in Boolootian, 1967). P. helianthoides were generally observed in all rocky sub-tidal areas covered by SCUBA diving exploration from 0 to 50 feet in depth. This animal produces a wide range of responses from other invertebrates. Sand Dollars (Dendraster sp.) burrow into the sand as it approaches scallops start to swim, ophiuroids, and other asteroids move away. The sea-cucumber, Parastichopus californicus exhibited a violent whipping and lengthening response when touched by P. helianthoides.

Leptasterias hexactis, the 6-rayed star, is a small olive-drab asteroid with 6 rays. It occurs intertidally on the Pacific Coast. It is an active carnivore feeding on turbinate snails, limpets, or almost any available animal food. The L. hexactis which were used in this study were collected intertidally around Victoria, the west coast of Saanich Inlet and San Juan Island. When Leptasterias hexactis is brought into close proximity with either of the two Pacific Coast abalones, Haliotis cracherodii or Haliotis rufescens, they will twist their shells through 180° several times and move rapidly away from the asteroid.

All of the animals, with the exception of Hippasteria spinosa, occur in many other areas. However I have found that in the above mentioned areas one can find these asteroids easily and repeatedly. After initial collection, the animals were placed quickly, but gently, into large containers of sea water

and transported to the laboratories. There, they were either dissected at once or held for future work in large tanks of fresh running sea water.

3. Fixation and Wax Embedding

Small pieces of tissue were removed from the aboral surfaces of all the above mentioned asteroids and placed into fixative solutions. The fixatives used were Heidenhain's S U S A (Pantin, 1962), Baker's formal-calcium (Pearse, 1961), and Bouins fixative (Carleton and Drury, 1957). A tisse sample from each animal, which was fixed in formal-calcium was used as a control and received no further chemical treatment. The remainder of the tissue was divided and placed in either Bensley's formic acid citrate decalcifier (Davenport, 1960), or in the calcium chelating agent EDTA (Ethylenediamine tetra-acetic acid)(Pearse, 1961). The Bensley's decalcifer was used in conjunction with routine staining for general histological examination because it tended to increase the staining properties of the tissue. The EDTA was used exclusively on tissues to be used for histochemical tests. After decalcification, the tissue was washed in distilled water and dehydrated by passing it through a series of 50%, 70%, 90% and 100% alcohols. Xylene was used as a clearing agent after which the tissue was infiltrated and embedded in Paraplast (M. P. 56° - 57° C.).

4. Fixation and Plastic Embedding

Tissue to be embedded in Epon 812 was removed from the aboral surface of the animal and quickly placed in the fixative, osmium tetroxide which was buffered with sodium bicarbonate (according to the technique compiled by Dr. P. Dudley in the embedding and staining notes supplied to workers at the Friday Harbor Laboratories, 1965). The buffer was prepared by making a 2.5% solution of sodium bicarbonate and regulating the pH to 7.2 with HCL. One part buffer was used with one part 4% OsO_4 to prepare the fixative. The pieces of tissue were placed in this solution for one hour at 0 C. in an ice bath, after which they were washed briefly in distilled water. Following this, dehydration was carried out as follows:

1. 30% EtOH - 8 min.
2. 50% EtOH - 8 min.
3. 70% EtOH - 8 min.
4. 95% EtOH - 10 min.
5. 95% EtOH - 2 min.
6. 100% EtOH - 5 min.
7. 100% EtOH - 5 min.

Following dehydration, the tissue was placed in propylene oxide (3 changes: 10 min., 10 min., and 5 min.) before infiltration with Epon 812 resin made up of 2 mixtures and an accelerator as recommended by Luft (1961). The details are:

Mixture A

1. Epon 812 - 62 ml.

2. DDSA (Dodecenyl succinic anhydride) - 100 ml.

Mixture B

1. Epon 812 - 100 ml.

2. NMA (Nadic methyl anhydride) - 89 ml.

Accelerator

DMP - 30 (2,4,6, - tri(dimethylaminamethyl) phenol.

The working resin was composed of 6 parts of mixture A and 4 parts of mixture B with 0.15 ml. DMP-30 to give 10 ml.

The specimen was placed into a 1:1 mixture of propylene oxide and the resin mixture for two hours. At the end of this time it was transferred to a 1:3 mixture of propylene oxide and the resin and left for three hours. The specimen was then transferred to a fresh mixture of pure resin and polymerized for 18 - 30 hours at 60°C. in No. 00 or No. 0 gelatin capsules.

Embedding in Epon 812 has one great advantage over the wax (Paraplast) embedding medium. Tissues embedded in Epon 812 show far less cellular distortion due to the embedding media than tissues which have been embedded in Paraplast. With wax embedding, the tissue damage is caused by the sudden, sustained heat of the molten wax when it is replacing the clearing agent and infiltrating the tissue. This imposes a very severe strain on the tissue as does shrinkage when the Paraplast solid-

ifies. However, the polymerization of Epon 812 requires that no antimedium be present. Very little heat, and shrinkage are produced by the polymerization process. Therefore the effects which produce swelling and shrinkage artifacts due to embedding are minimized in the Epon 812 embedded tissue when compared to the wax embedded tissue. Fixatives for paraffin embedding must prepare the tissues to withstand heating and often this treatment detracts from the details of cellular structure. Fixatives such as Osmium Tetroxide, which are excellent for the retention of delicate structures do not prepare the tissue for the strain of molten paraffin and cannot be used successfully to show cellular details in this type of embedding (Baker, 1950). However, they may be used with the less rigorous treatment of embedding with Epon 812 to great advantage in light microscopy. Moreover, the tissue embedded in Epon 812 suffers far less distortion in the sectioning process than the tissue embedded in Paraplast because the greater hardness of the Epon 812 provides more physical support to the tissue during the cutting process.

There are some less advantageous features of Osmium Tetroxide fixation and embedding with Epon 812. Glass and diamond knives only are sharp enough to successfully cut this material. To obtain quality sections, a special, or ultramicrotome is required. Also, due to the poor penetrating qualities of Osmium Tetroxide only small pieces of tissue, not over 1 mm. thick, can be successfully penetrated by the fixative, unless dealing exclusively with surface structures. In spite of these disadvantages, the Epon 812 and the plastic embedding materials

in my experience are generally vastly superior to other embedding media due to the great detail which they reveal in tissues that have been successfully infiltrated.

5. Sectioning

A. Paraffin sections

The tissue embedded with Paraplast was cut at 6 - 20 µ sections on a Spencer AO "820" microtome using a Spencer microtome knife. The ribbons cut from the tissue were flattened by floating them on the surface of a water bath heated to just below the melting point of the Paraplast, and which contained a small amount of dissolved gelatin to assist the later attachment of the tissue to the slide. The flattened sections were separated from the ribbon, picked up on a slide, and left to dry after rolling with a rubber roller to insure the best possible contact between the tissue and the slide.

B. Epon 812 sections

The calciferous material embedded in Epon 812 was sectioned on the Sorvall Porter-Blum ultra-microtome MT-1, for thick 1-3 µ sections for use with the light microscope. The Sorvall Porter-Blum motorised ultra-microtome MT-2 was used for thin 700 - 1000 Å sections to be studied with the electron microscope. In both cases the calcium deposits in the tissue required the use of a diamond knife because glass knives

are not sufficiently hard to cut through these deposits and remain undamaged. The cutting angle of the diamond knife (Dupont) was 46°.

The sections produced were floated on distilled water. Those sections to be viewed with the light microscope were lifted from the surface of the water with a hair or a thin wire loop and placed on a clean glass slide. The slide containing the section was then set on a heated metal plate to evaporate the water and cause the Epon 812 to adhere to the glass slide. Those sections for examination with the electron microscope were removed by pressing a 200 mesh, carbon coated grid against the section floating on the surface of the water. The sections then adhered to the grid when it was lifted from the water and were placed in a clean container to dry without contamination by air born dust.

C. Frozen sections

Bakers formal-calcium was used as a fixative for this tissue to reduce lipid extraction. The tissue was then decalcified in EDTA washed, and embedded in gelatin (Pantin, 1962). Sections were cut either on a Coon's type cryostat (Harris Manufacturing CO.) or on a freezing microtome (Sartorius - Werke Model 27), cooling being produced by a freezing stage (Lipshaw thermo-electric section freezing stage, Model 295). In both cases the sections were placed in water and picked up on gelatinized slides which were exposed to formalin

vapors to complete attachment.

6. General Staining

A. Paraffin Section Staining

General staining was done on paraplast embedded sections in order to study the gross structure of the epidermal tissue. Two stains were used: Masson's Trichrome (Pantin, 1962); and One Step Mallory (Simpson, 1950, cited in Emmel and Cowdry, 1964).

B. Epon 812 Thick Section Staining (light microscope)

Unlike embedding wax, Epon 812 cannot easily be removed from the tissue. This imposes restrictions on the stains that can be used. Most common stains or staining mixtures will either stain the Epon 812 itself, or will not stain the tissue at all. I found Richardson's stain to be the most successful stain for tissue embedded in Epon 812. The Richardson's stain was superior to other Epon 812 stains in providing good contrast and distinctly showing the delicate internal structures of nearly all cells. It is composed of a 1:1 mixture of 1% Azur II in distilled water and 1% Methylene Blue in 1% borax (Richardson et al. 1960). The staining method was as follows:

1. 1% Periodic acid - 5 min. (optional)
2. Running water - 1 min.
3. Dry on warming plate.
4. Cover the warm slide with stain. 10-60 sec.

5. Rinse with water - 2-3 min. (Important)

6. Examine while wet for metachromasia.

7. Dry on warming plate and mount in immersion oil.

The Richardson's stain demonstrates the tissue structure very well and the Epon 812 remains colorless. It is best viewed and photographed with a thin film of immersion oil under a size 1 1/2 coverslip.

C. Epon 812 thin section staining (Electron Microscope)

The sections for use in the electron microscope were picked up and dried on 200 mesh grids that had been carbon coated. Aqueous 2% Uranyl Acetate (Watson, 1958) was the staining solution. The staining was carried out for two hours in the dark, and, when observed in the Philips EM 100B at 60 KV using a 25 μ objective aperture, proved to be a good general stain.

7. Histochemical Tests

The histochemical tests were carried out on tissue embedded in Paraplast and gelatin. These results were correlated as closely as possible with the same structures seen in tissue embedded in Epon 812 to gain some basic chemical information about the various gland cells observed in the different asteroids studied. Sixteen histochemical tests were tried on the tissues taken from the aboral surfaces of the eight asteroids. The results of these tests are presented in

full in Appendix I.

A. Periodic Acid-Schiff Reaction (P.A.S.)

The P.A.S. reaction (cited in Pearse, 1961, after McManus, 1946) was used to determine the presence of new aldehydes formed after limited oxidation with periodic acid. A positive response indicates the likely presence of polysaccharides, neutral mucopolysaccharides, muco- and glycoproteins, glycolipids or phospholipids. In some cases, other types of histochemical tests used in conjunction with the P.A.S. reaction can separate the above substances. The Schiff's reagent used in the P.A.S. test was prepared by the Barger and DeLamater method (1948) as described in Pearse (1961). All tissues tested for the P.A.S. reaction were also tested without the initial treatment by periodic acid to check the possibility of naturally occuring aldehydes in the tissue which would produce a false reaction.

B. Detection of Acid Mucopolysaccharides

Several methods were used to detect the presence of acid mucopolysaccharides in the gland cells: The modified Hale Colloidal Iron method as given in Barka and Anderson, 1963 was used in conjunction with sections treated only with potassium ferrocyanide-hydrochloric solution to prevent false results due to any intrinsic iron in the tissue; the Alcian Blue 8GX (Steedman 1950, cited by Pearse, 1961) reaction; the Methylene Blue Extinction test (Dempsey and Singer, 1946, cited

by Pearse(1961), and the Acridine Orange test (Hicks and Matthaei,(1958) cited by Pearse 1961). In the last method, the acid mucopolysaccharides fluoresce a brilliant reddish-orange color, whereas the fluorescence of most other tissue components is quenched. The fluorescent light for this test was produced by an Osram HBC 200 high pressure lamp unit. A 2 mm. Schott BG 12 exciter filter was used to give a wave length of 250-500 mμ (Culling, 1963) which was passed to the specimen through the quartz optics of a Reichert Binolux microscope.

C. Metachromatic Reactions

A staining reaction is classified as metachromatic when a dye stains a structure a color which is different from a dilute solution of the dye. In this study, the Toluidine Blue method (standard) as given in Pearse(1961)was used to show metachromatic substances. In this case the metachromatic substances are shown a red-purple color, and the orthochromatic substances are shown a blue color.

D. Dihydroxy Dinaphthyl Disulfide (D.D.D.) Reaction

This is a highly specific reaction for -SH groups derived from the fact that only the thiol groups in proteins are oxidized by disulfides at alkaline pH. The DDD compound (2,2-dihydroxy-6,6-dinaphthyl disulfide) reacts with the -SH groups of fixed proteins at alkaline pH (8.5) to form a complex that is insoluble in water and organic solvents. After proper washings to remove the unused reagent and by-products

(6-thio-2-naphthol), a colored azo dye is developed by coupling with a diazonium salt (Barka and Anderson, 1963).

E. Ninhydrin Method

In a neutral medium ninhydrin reacts with the ∝-amino acids of proteins. Ninhydrin oxidizes ∝-amino acid to imino acid, which decomposes to a keto acid (spontaneously or by heat) to form aldehyde, carbon dioxide, and ammonia. The new-formed aldehyde groups are demonstrated by Schiff's reagent (Baka and Anderson, 1963).

F. Lipid Detection

The detection of lipids requires two distinct operations in order to confirm the presence of these compounds in the tissue. The first is the use of a coloring agent, called a lysochrome, which is more soluble in lipid than it is in the solvent in which it is dissolved. The second is the extraction of the lipid by solvents. This is confirmed by the use of lysochromes.

I was not able to complete the second part of this operation, and therefore can only suggest the possibility of gland cells which contain lipid.

The following tests were employed to examine gland cells for the presence of lipid on the aboral surface of the asteroids studied: Sudan Black B (Liston, 1943, cited in Barka and Anderson, 1963); Oil Red O (Lillie 1944, cited in Pearse, 1961); Sudan IV

(Kay and Whiteheah, cited in Pearse, 1961); Phosphene GN (Popper, 1944, with Phosphene 3R, cited in Pearse, 1961); and 3:4 Benzpyrene (Berg, 1961, cited in Pearse, 1961).

Acetone-Sudan Black B (Berenbaum, 1958, cited in Pearse, 1961) and the Burnt Sudan Black B method (Berenbaum, 1958, cited in Pearse, 1961) were used to test for bound lipids in paraffin sections.

The Sudan Black B method for masked lipids (Ackerman, 1952, cited in Pearse, 1961) for blood smears was also tried.

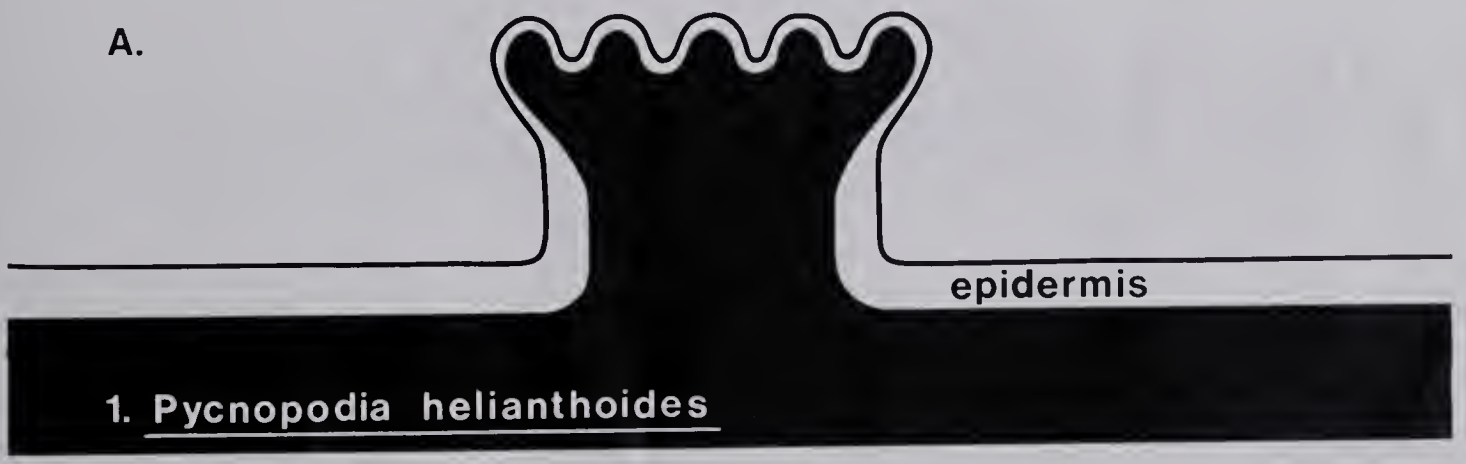
The Nile Blue Method (Menschik, 1953, cited in Pearse, 1961) was used to indicate the presence of phospholipids.

III. RESULTS

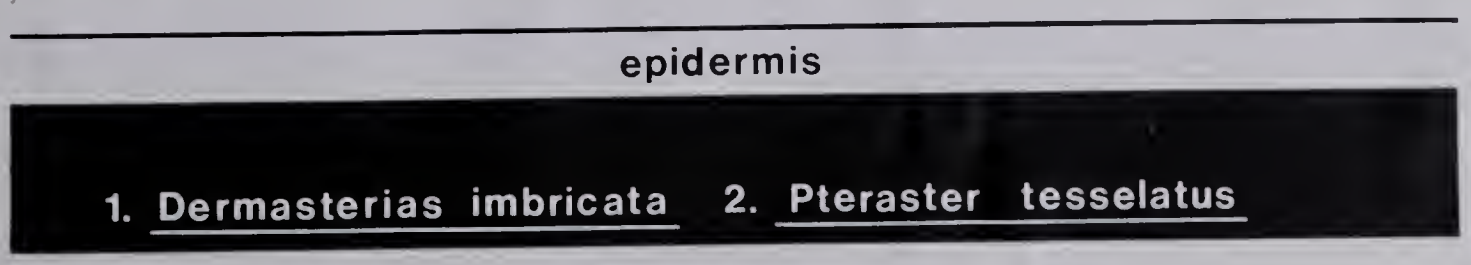
1. General Structure of the Asteroid Epidermis

There is considerable variation in the nature and extent of the epidermal covering and associated structures on the aboral surface of asteroids. Before presenting the detailed histological and histochemical results from the asteroids examined in this study, it will be desirable to describe their general structural features. The following drawings will illustrate some of the terminology applied to the asteroid epidermis and show the position of the epidermis proper in relation to various structures:

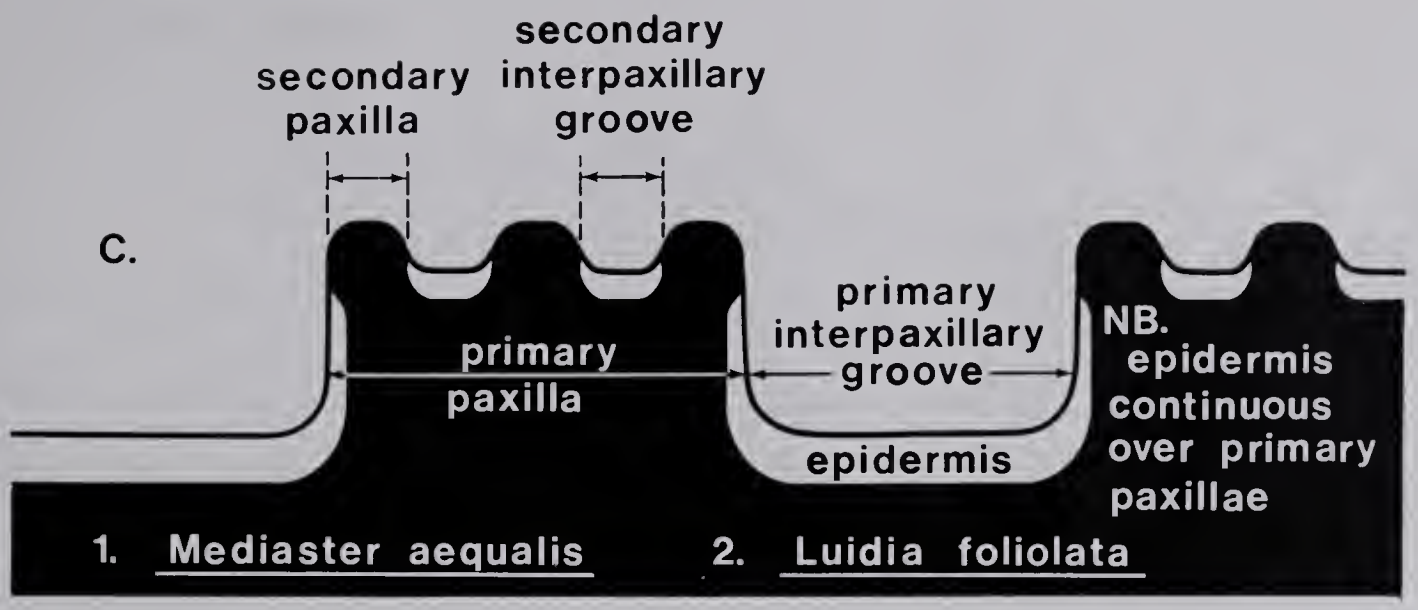
A.



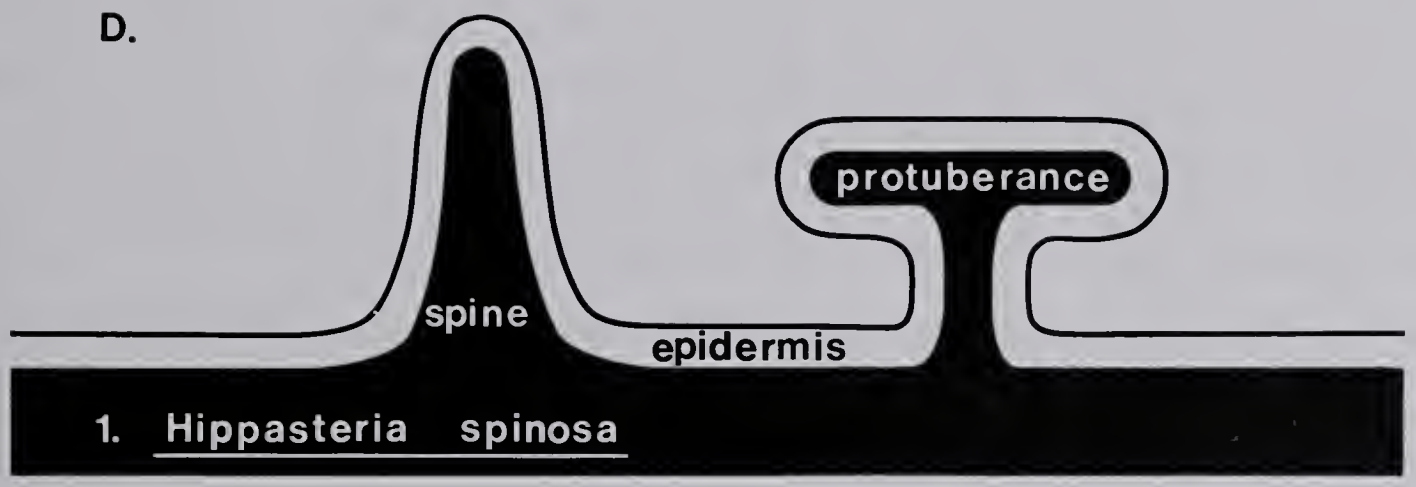
B.



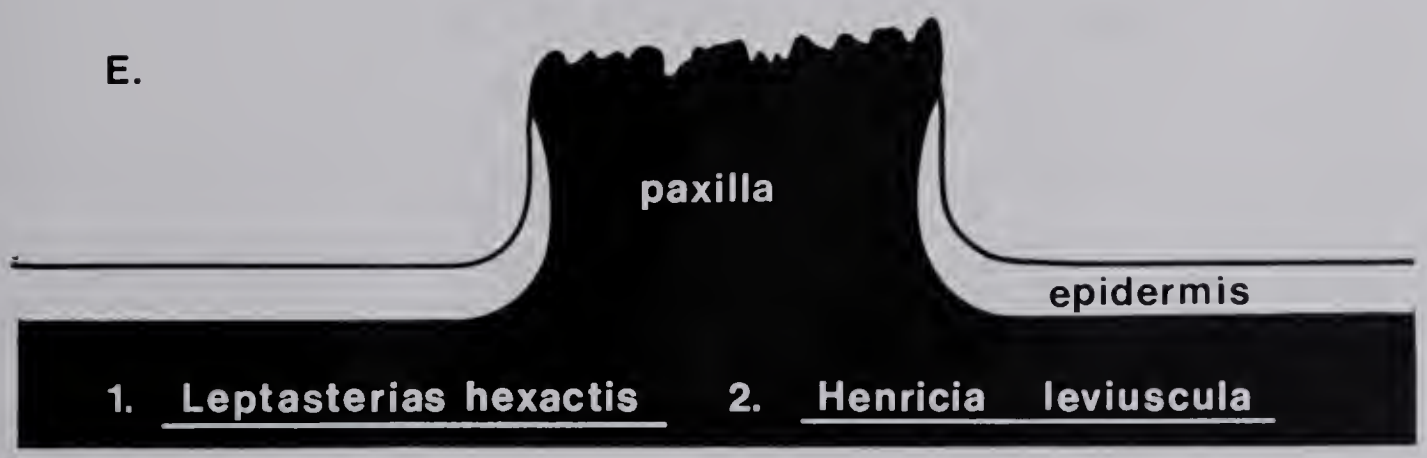
C.


secondary paxilla
secondary interpaxillary groove
primary paxilla
primary interpaxillary groove
epidermis
NB. epidermis continuous over primary paxillae
1. Mediaster aequalis
2. Luidia foliolata

D.


protuberance
spine
epidermis
1. Hippasteria spinosa

E.


paxilla
epidermis
1. Leptasterias hexactis
2. Henricia leviuscula

2. Detailed Results

A. Dermasterias imbricata

On the aboral surface of Dermasterias imbricata (fig. 1) there is an epidermal layer about 60 μ deep. This layer covers the entire surface of the animal, with the exception of dermal branchiae where it is reduced. No calcium spicules are present in this layer so that the animal to the touch has a very slick, smooth surface. This asteroid has no cilia on its aboral surface. The structure of the epidermis of D. imbricata is unique among the asteroids included in this study in that no structure resembling a basement membrane was observed at the base of the epidermal layer (Fig. 3, Fig. 6). After osmium tetroxide fixation and Epon 812 embedding the Richardson's staining showed no distinct cellular outline in the epidermis except gland cells (Fig. 5, Fig. 6). These gland cells take the form of distinct entities which appear to sit in a relatively amorphous matrix occuring below and around the gland cells. The electron microscope, however, shows that this amorphous area is composed of distinct cells exhibiting typical cellular organelles (Appendix II). The light microscope shows the surface of these epidermal cells to be capped by a structure resembling a cuticle 1.8 μ deep which appears to have a thin, dense surface layer and a less dense but thicker layer below (Fig. 5). The electron microscope shows this cuticle-like layer to be composed of an amorphous material, probably secreted by the underlying

epidermal cells, which exhibit three bands of varying density (Appendix II). This structure is penetrated by a network of tubules. These tubules apparently connect the interior of the cell with the external environment. Some evidence of secretion is evident at the surface of the pore (Appendix III). For convenience these tubules will be called cellular projections.

Two distinct gland types are present in the aboral epidermis of D. imbricata. The first and most common, which will be called Type A, was first generally described in paraffin sections by Ward in 1965. It is a single cell containing acid mucopolysaccharide (Table I) (Fig. 2). The second type of gland cell in the same position in the epidermis will be called Type B, it was not described by Ward. Type B (Table I, Figs. 5, 6) generally resembles Type A with the exception that it is smaller, less frequent in distribution, and does not contain acid mucopolysaccharide, but a P.A.S. positive compound suggesting neutral mucopolysaccharide. The entire epidermal area, and aboral tissue in general is strongly P.A.S. positive. A third tissue component which appeared to be glandular was seen in D. imbricata but not in any of the other asteroids studied. They were first observed by Ward in 1965 who called them "dermal spherules". Because they are true cells, I prefer to call them "dermocytes". The dermocytes appear as a series of spherical, nucleated cells in bead-like columns, stacked in single rows one above the other at right angles to the dorsal surface of

the animal (Table 1, Figs. 3,4). These columns are separated from one another by connective tissue and collagen fibers. They appear to be less spherical and less vacuolated at the base of the column and progressively become more spherical and more vacuolated toward the top of the column, suggesting a progression of development from the bottom to the top. However there is no evidence that these cells are discharged from the animal nor have any ducts been seen in conjunction with these cells by which their contents might be liberated to environment. These structures appear to have large numbers of -SH bonds as shown by the positive D.D.D. reaction indicating a high protein content. This has been tentatively verified by the ninhydrin reaction.

TABLE I

COMPARATIVE DATA ON THE ABORAL GLAND CELLS OF DERMASTERIAS IMBRICATA

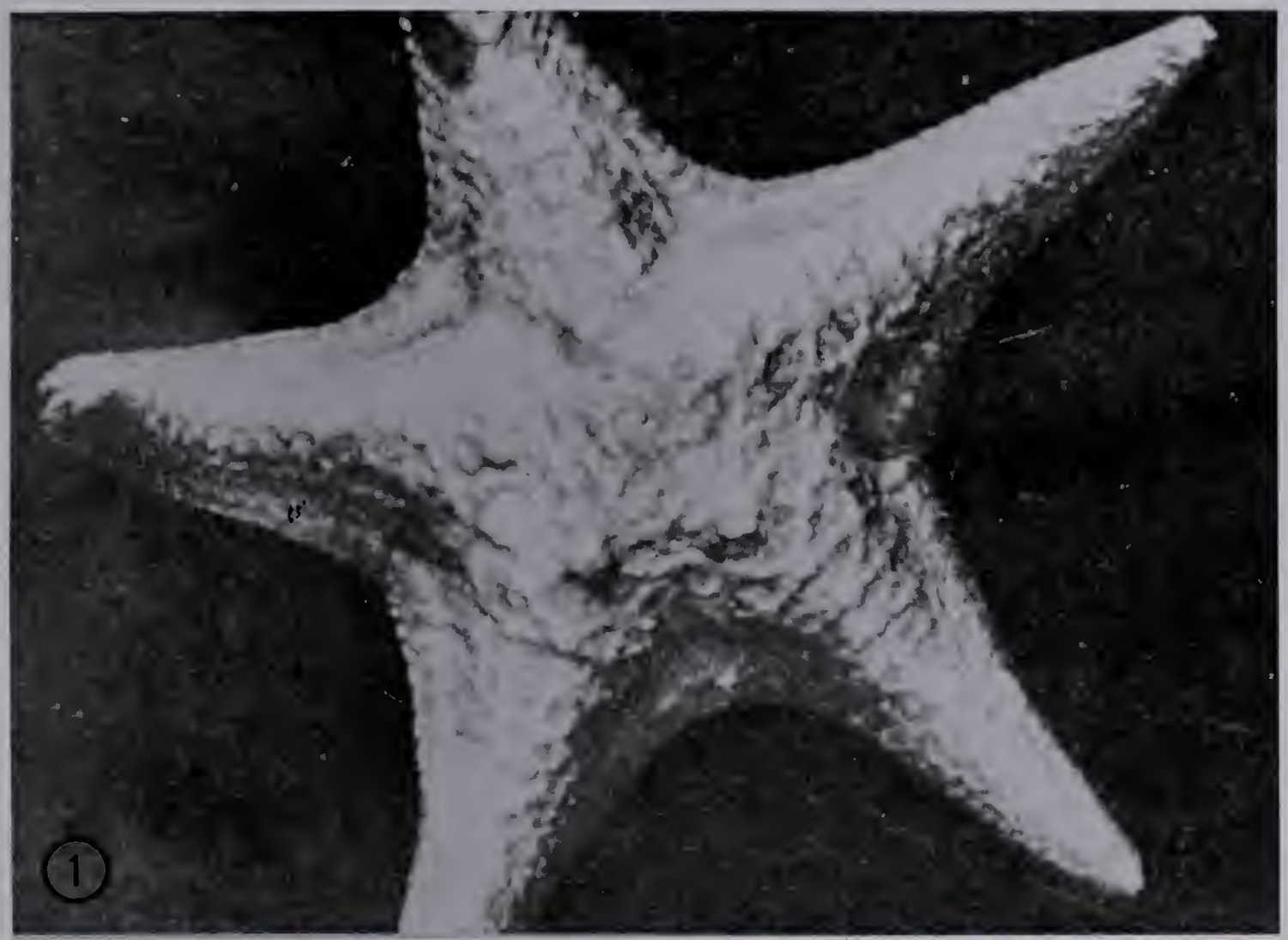
		Gland Type A	Gland Type B	Dermocytes
length		60 μ	60 μ	15 μ
width	base	7 μ	2 μ	15 μ
	neck	2 μ	1 μ	15 μ
secretory particle diameter		3 μ	0.6 μ	1.5 μ
chemical composition of gland cell		acid mucopoly-saccharide	P.A.S. +	protein
opening to exterior		yes - pore	yes - pore	no
approximate ratio in epidermis		60%	40%	0%
location of nucleus		base	base	central
distribution in epidermis		entire	entire	below entire epidermis
Remarks				formed in columns

Fig. 1. Photographic reproduction of the asteroid Dermasterias imbricata from the original picture by Fisher, 1911. X ½.

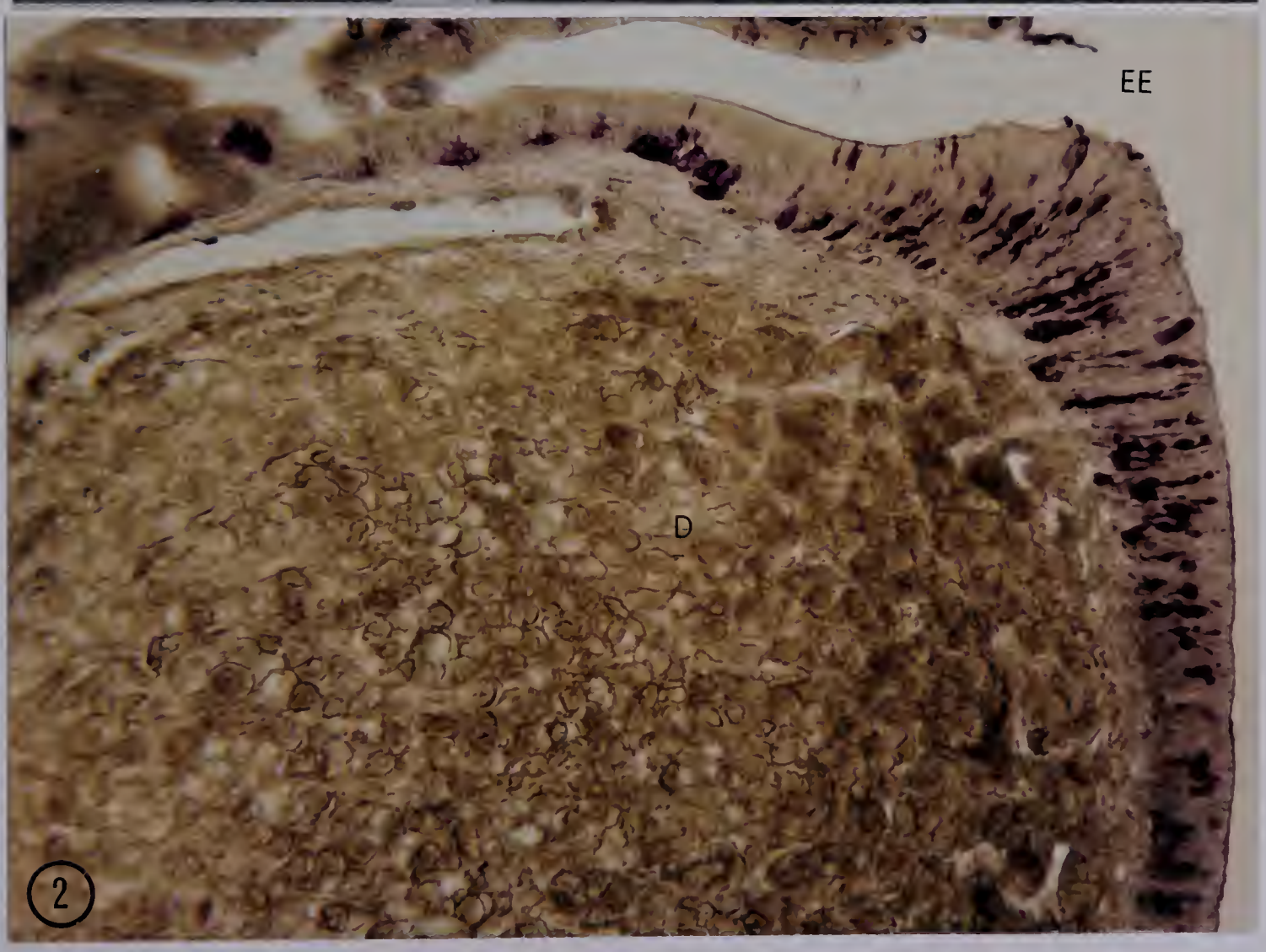
Fig. 2. Photomicrograph showing the surface aboral tissue of Dermasterias imbricata. The purple, metachromatic stained areas are Type A gland cells. No other tissue is stained.

D, dermocyte layer; EE, external environment.

SUSA Fixation Paraplast embedding, 10 u thick, stained with Methylene Blue, pH 3.



1



2

Fig. 3. Cross section through the aboral epidermal tissue of Dermasterias imbricata.

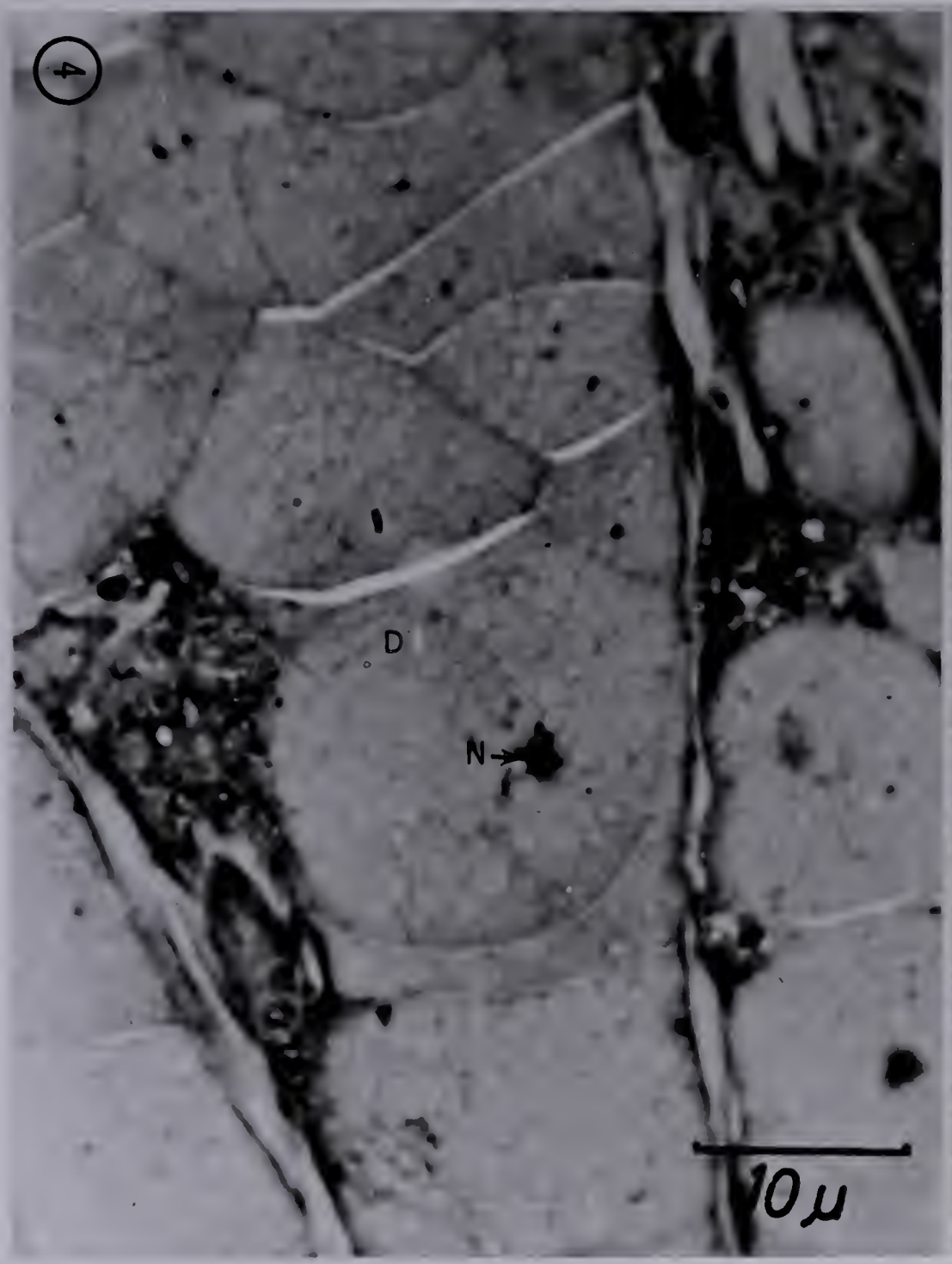
Cu, cuticular layer; D, dermocyte layer; EE, external environment; Ep, epidermis.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's staining.

Fig. 4. Dermocytes showing typical vacuolated cellular appearance.

D, dermocyte; N, nucleus.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's staining.


4
D
N
10μ

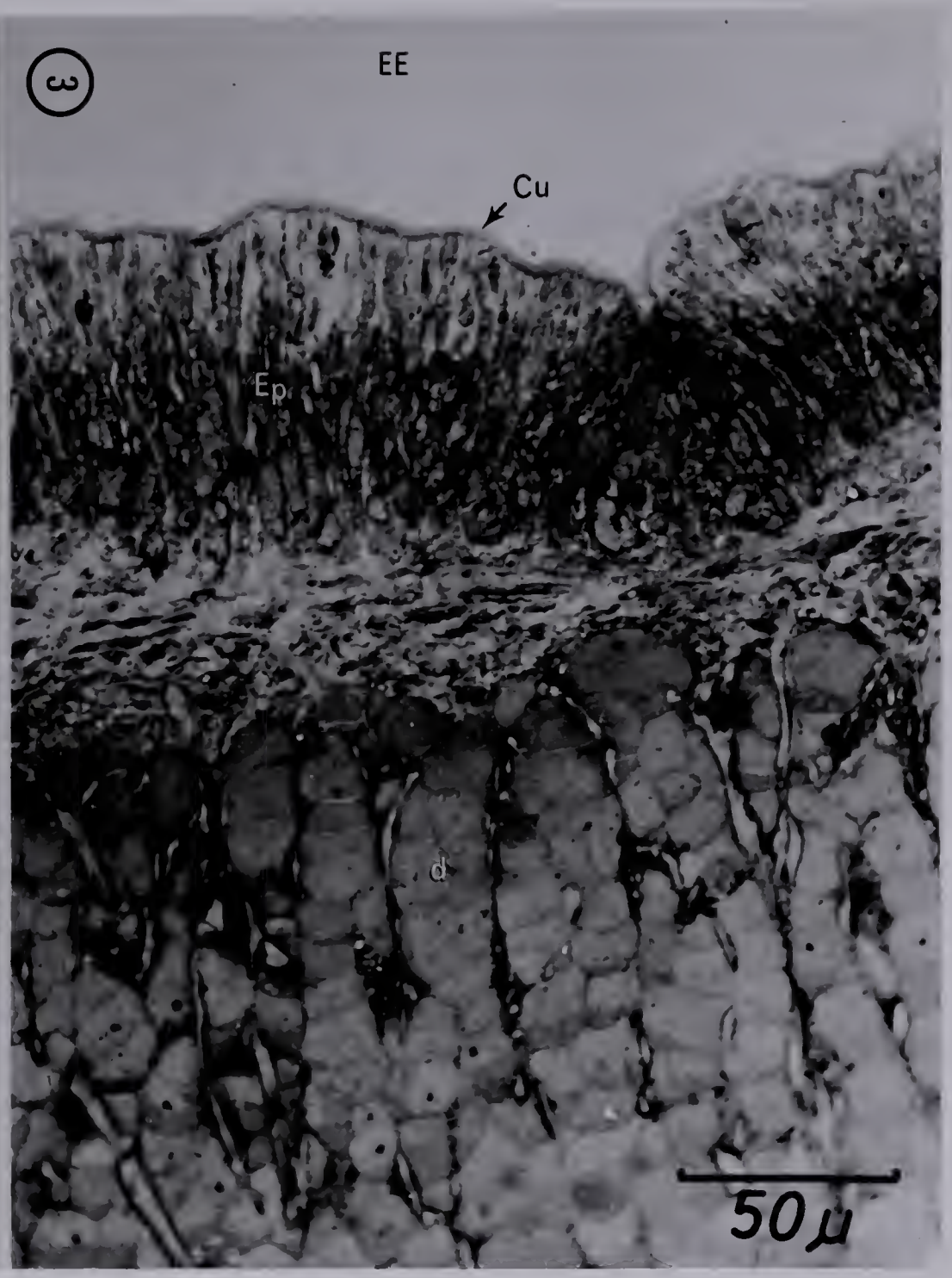
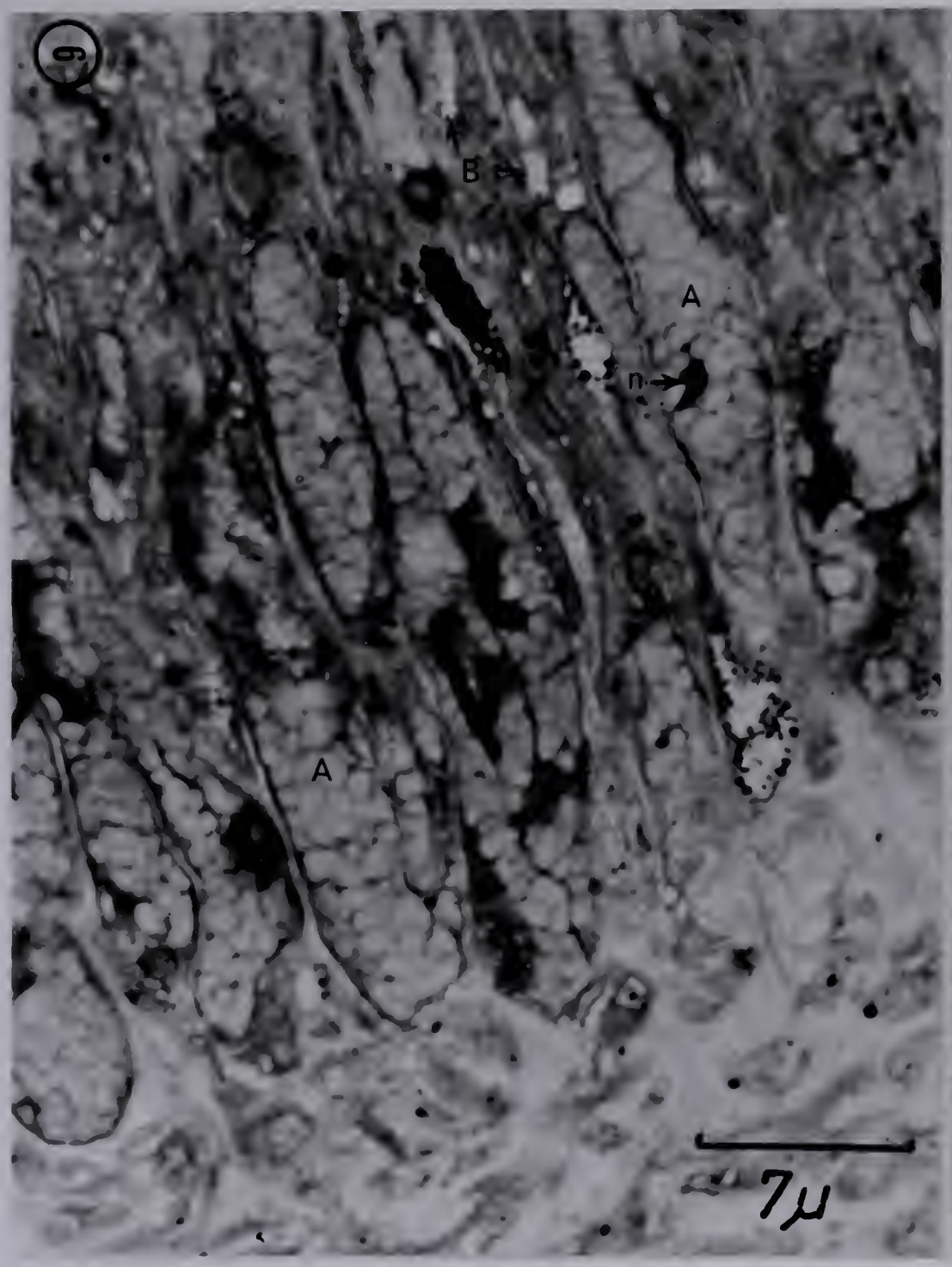

3
EE
Cu
Ep
d
50μ

Fig. 5 & 6.These two photomicrographs show the epidermal structure of Dermasterias imbricata. Fig. 5 shows the top half of the epidermis, and Fig. 6 the bottom half of the same area. Note the apparent lack of a basement membrane in Fig. 6.

A, Type A gland cells; B, Type B gland cells; Cu, cuticle; EE, external environment; N, nucleus.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's staining.


6
B
A
n
A
7μ


5
EE
cu
B
A
7

B. Luidia foliolata

L. foliata has an aboral surface which is composed of large primary paxillae which contain numerous secondary paxillae. Over the entire aboral surface of the animal is an epidermal layer of varying depth (Page 23,C). In the interpaxillary groove of the primary paxillae the epidermis reaches a maximum depth of 70 μ where as on the top of the secondary paxillae it may be as shallow as 15 μ. The structure of the epidermis is vague, due to the fact that Epon 812 embedding and the sections treated with Richardson's stain show only scattered nuclei with no distinct cellular boundaries. The only distinct cellular tissue appear to be the gland tissue (Fig. 9). The epidermis does not appear to have a cuticular structure covering its surface, but in the light microscope it appears to have a layer of microvillae, of uneven length covering the surface. Flagella are scattered over the surface of L. foliolata but appear to be more prominent in the interpaxillary grooves than on the surface or sides of the paxillae. In the thick epidermis of the primary paxillary groove a dark, granular pigment was observed (Fig. 12). The pigment is in the form of dense black-brown granules 2.5 μ in diameter which give the appearances of being loosely held in distinct areas. The pigment granules occur in the lower two-thirds of the epidermal layer but may on occasion fill the entire area. The pigment itself may be melanin. It is highly resistant to all chemicals, but it can be bleached by hydrogen peroxide. Below this pigment layer invariably occurred an unusual non-nucleated fibrous tissue of

unknown function (Fig. 11).

Two distinct types of glandular cells are present in the aboral epidermis of L. foliata.

The glandular cells to be called here Type A (table 2) occurred only in the epidermis enclosing the top surface and sides of the secondary paxillae. The structure of this cell appeared to be vastly different from all the other asteroid glands observed to date. Its body appeared to be composed of a long tube, or perhaps a series of connected tubes, which twists and doubles back on itself in the base of the epidermal region. This gives the appearance of complex knots of gland tissue scattered in specific areas in the epidermis (Fig. 9). This series of gland tubes occasionally emerges at points (Fig. 10) where the products are presumably released to the environment. The interior of this gland contains secretory packets which histochemistry has shown to contain acid mucopolysaccharide (Fig. 13). Epon 812 embedded step sections will have to be taken through the Type A gland cell in order to obtain its proper physical structure.

Gland cells of Type B (Table 2) are smaller, but are more uniformly dispersed throughout the epidermis than the Type A gland cells. These cells produce secretory packets usually found in the upper half of the cell (Fig. 9). Directly below the secretory packets a dense cone shaped structure of an unknown function occurs, the base of which impinges on the sec-

retory packets (Fig. 12). When this type of gland cell occurs in the deep epidermis of the primary interpaxillary groove, the gland cell body takes on a very long, stretched appearance (Fig. 12). A secretory pore, or any means of releasing the secretory packets to the exterior environment has never been observed. The gland cells of Type B did not respond to any general stain or histochemical test used in this study, and have only been observed in Epon 812 embedded sections.

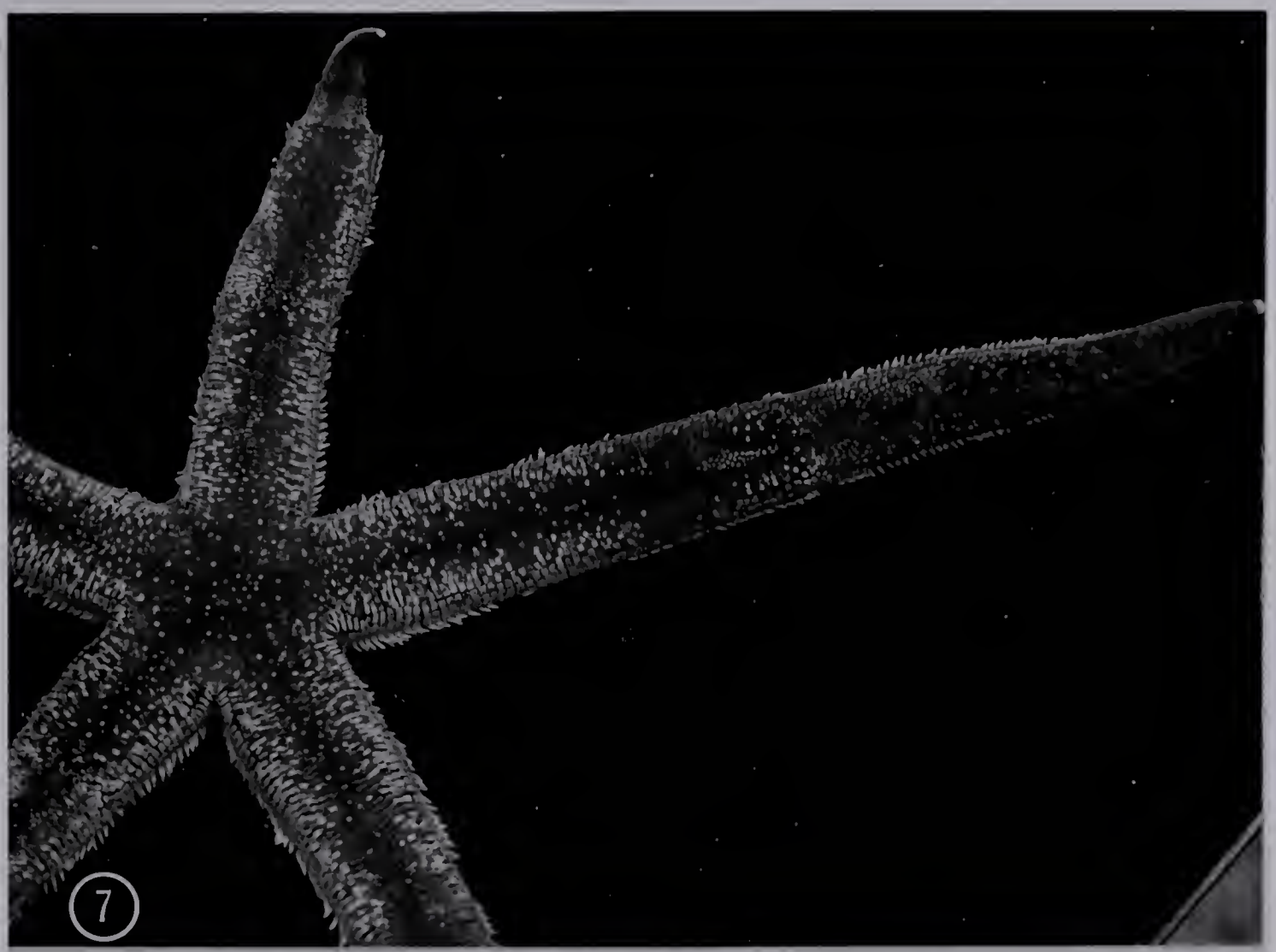
TABLE II

COMPARATIVE DATA ON THE ABORAL GLAND CELLS OF LUIDIA FOLIOLATA

		Gland Type A	Gland Type B
length		unknown	10 μ - 70 μ
width	base	8 μ	7 μ
	neck	8 μ	--
secretory particle diameter		up to 1 μ	.5 - 1 μ
chemical composition of gland cell		acid mucopoly-saccharide	unknown
opening to exterior		yes - pore	no
approximate ratio in epidermis		40%	60%
location of nucleus		unknown	below secretory packets
distribution in epidermis		top and edge of secondary paxillae	entire epidermis
Remarks			

Fig. 7. Luidia foliolata. Photograph taken from dried specimen. X ½.

Fig. 8. View of paxillae on aboral surface of Luidia foliolata. X ½.


7

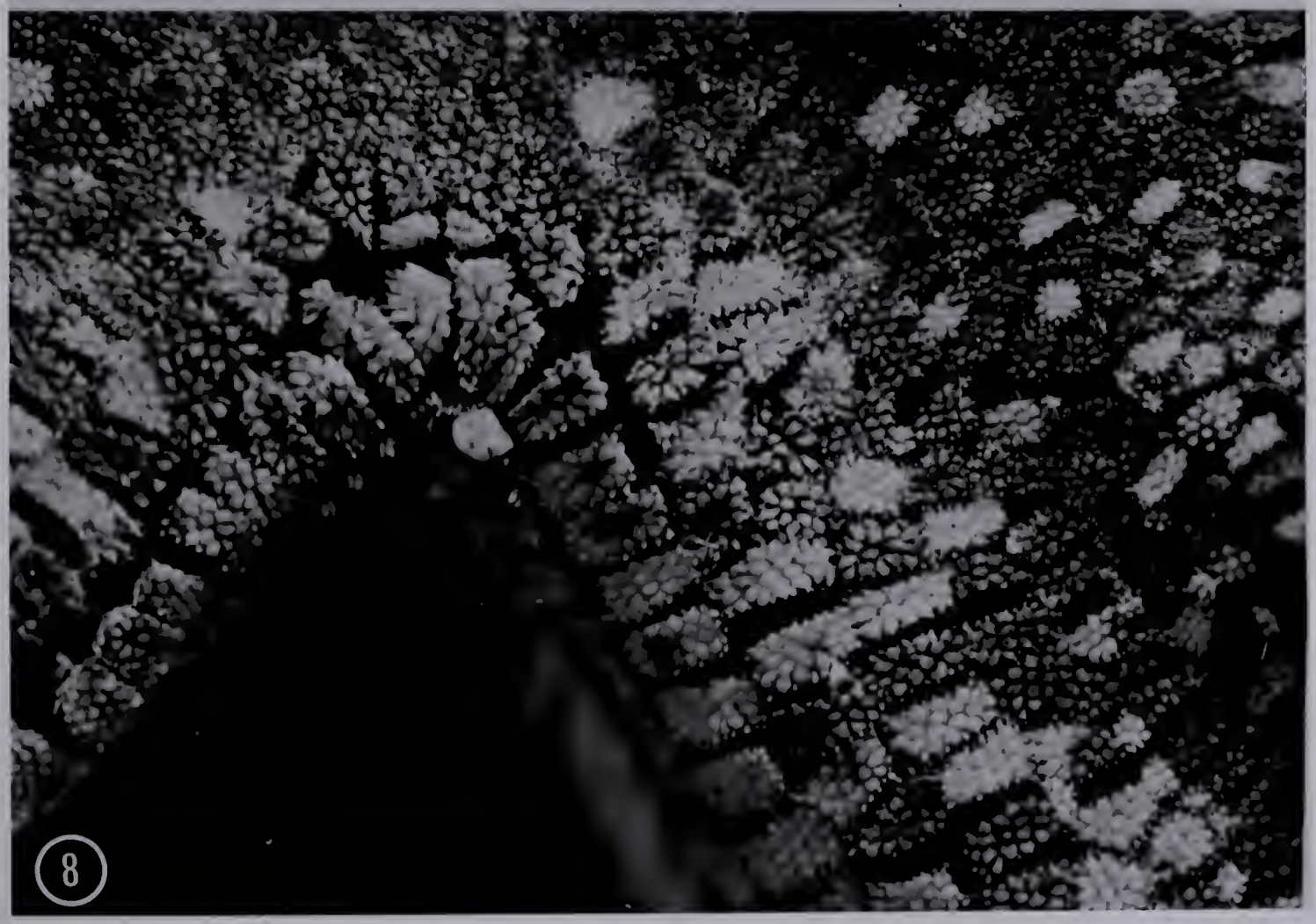

8

Fig. 9. Cross section through the epidermis at the top of a secondary paxilla showing the two different types of glands occurring in Luidia foliolata.

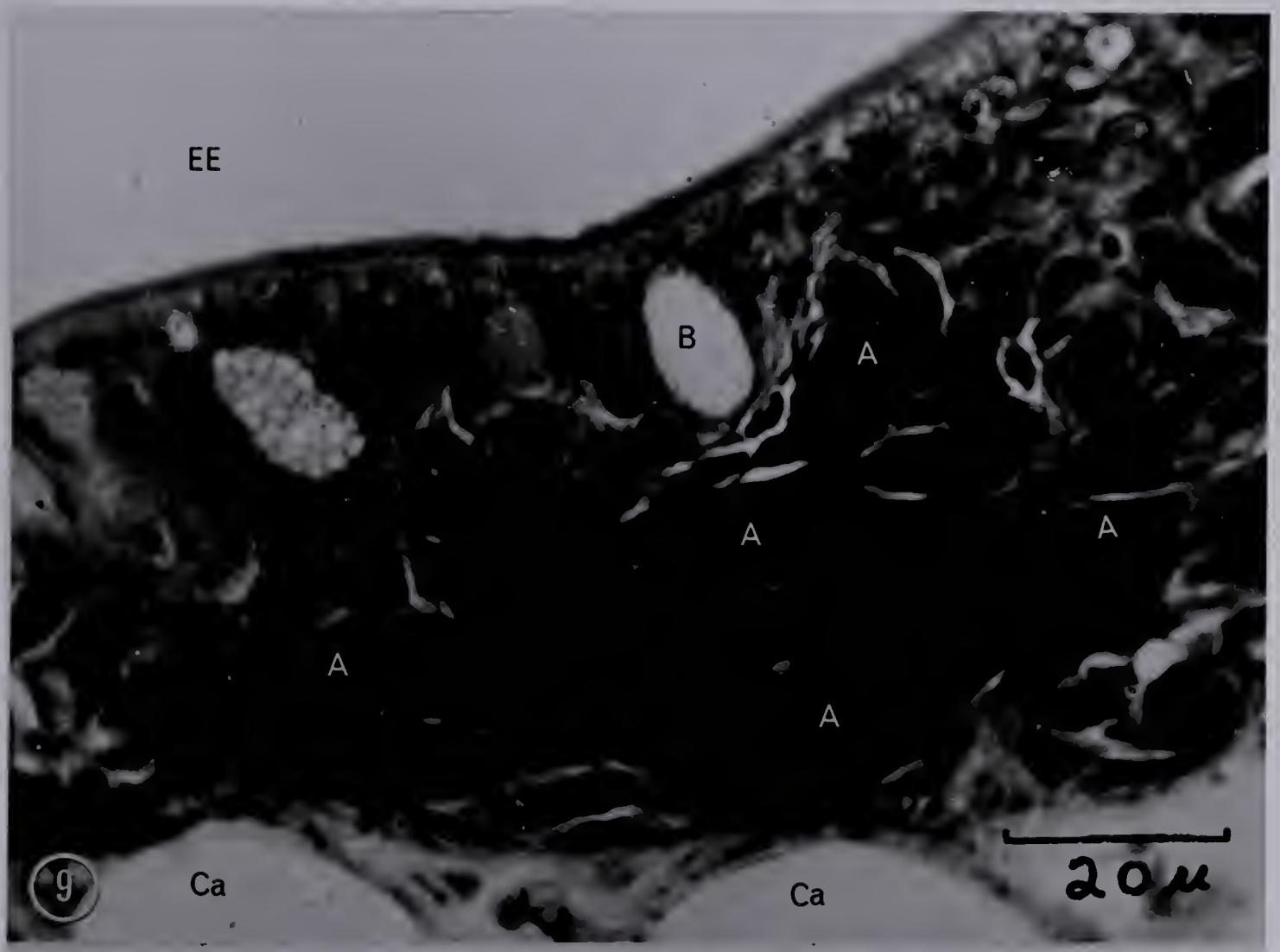
A, Type A gland cell tissue; B, Type B gland cell; Ca, site of calcium spicule; EE, external environment.

Bicarbonate;osmium fixation, Epon 812 embedding, and Richardson's staining.

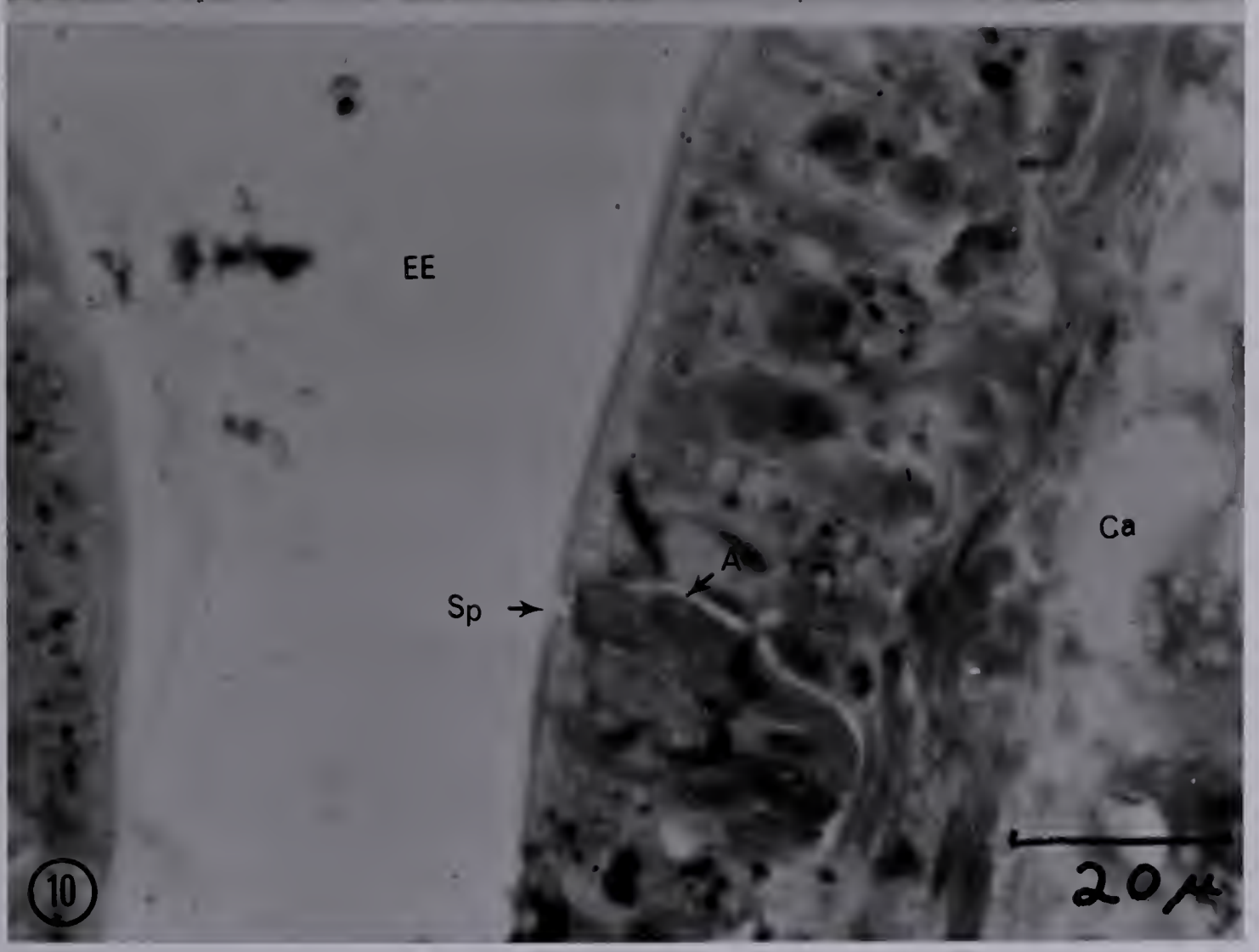
Fig. 10. Cross section through the upper edge of secondary paxilla which shows a secretory pore for Type A gland tissue.

A, Type A gland tissue; Ca, site of calcium spicule; EE, external environment; Sp, secretory pore.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's staining.



9



10

Fig. 11. Cross section of epidermal tissue in the primary inter paxillary groove on the aboral surface of Luidia foliolata.

B, Type B gland cells; Bm, basement membrane; Ci, cilia; EE external environment; Fl, fiberous layer, Pg pigment granules.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's staining.

Fig. 12. Higher magnification of an area similar to that shown in Fig. 11. Note the long cell bodies of the Type B gland cells in this area.

B, Type B gland cells; EE, external environment; F, long body of Type B gland cell; Pg, pigment granules.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's staining.


12
EE
B
f
Pg
f
15 µ

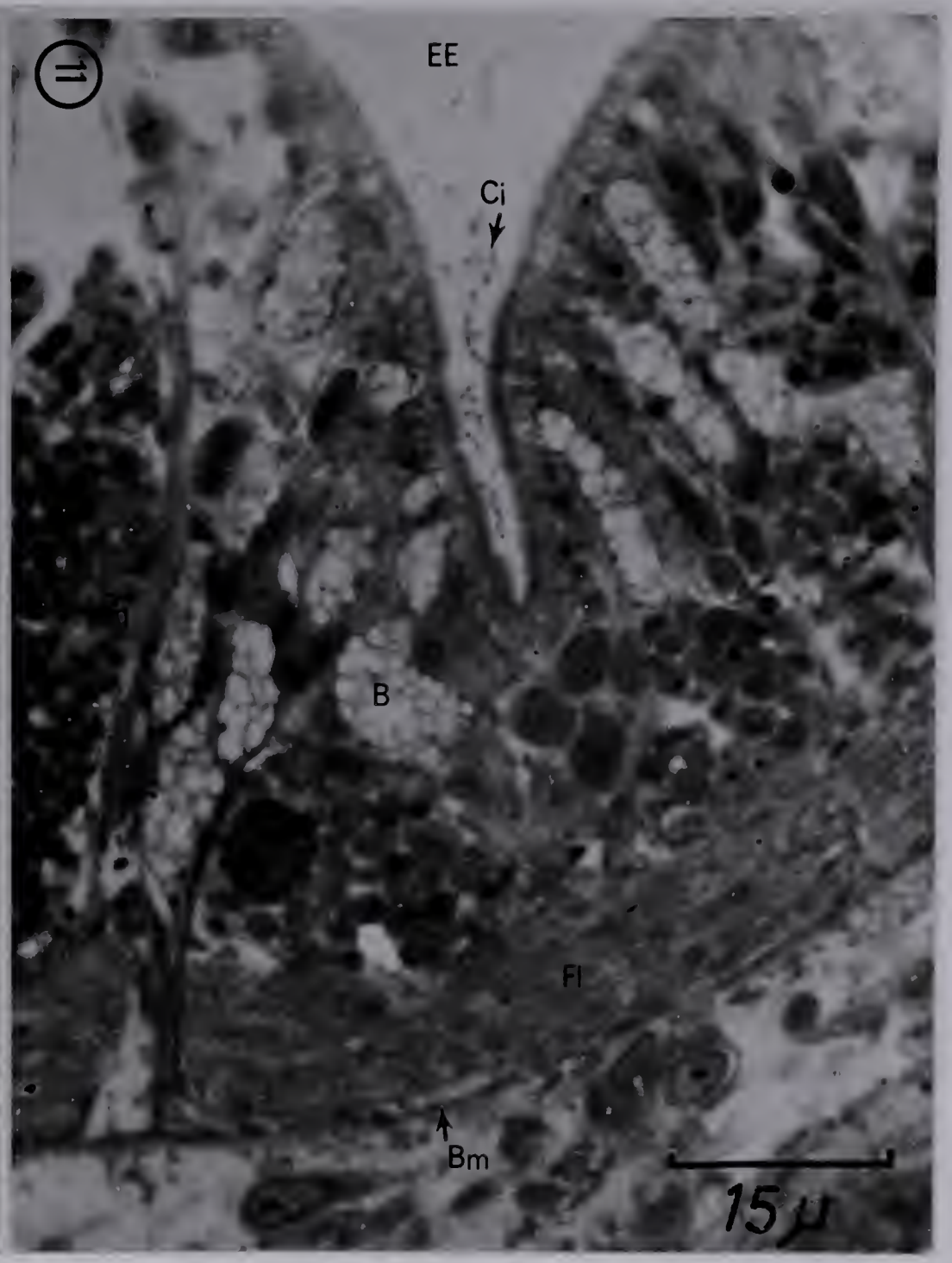

11
EE
Ci
B
Fl
Bm
15 µ

Fig. 13. Secondary paxillae on the aboral surface of Luidia foliolata showing Acridine Orange fluorescence in the type A gland tissue. Note that the glandular tissue is concentrated near the bottom part of the epidermis. SUSA Fixation, Paraplast embedding, Acridine Orange staining.

C. Mediaster aequalis

On the aboral surface of Mediaster aequalis (Fig. 14) the top of the paxillae have small round protrusions which form a rosette (Fig. 15) (Page 23 C). The epidermis is sparsely flagellated, and varies in depth in different regions. The deepest epidermis is found in the primary interpaxillary grooves. It is composed of columnar epithelium interspersed with gland cells and it has a uniform cuticle covering its dorsal most part, the epidermis rests onadistinct basement membrane. As this epidermis approaches the base of a paxilla it gets progressively thinner. This continues until the upper sides and top of the paxilla is covered mainly by the cuticle. This means that the top of the paxilla, the rosette structures, have little apparent epidermis (Fig. 18). These structures are heavily calcified. The calcium endoskeleton supporting the rosette takes the form of inter-connected ovoid bodies, orientated linearly at right angles to the plane of the dorsal surface, (Fig.18). Undefined tissue and prominent gland cells occur in the small space between the spicules (Fig. 19).

Three types of gland cell have been observed in the aboral epidermis of M. aequalis.

Cells of the gland type A (Table 3) occurred in the deep epidermis of the interpaxillary groove and on the base of the paxillae (Fig. 16). Their nuclei were located at the base,

and their cytoplasm filled with secretory vesicles. The gland cell opens to the surface via a secretory pore. Histochemically the Type A gland cell shows a light, but positive response to the P.A.S. reaction and an apparent positive response to the D.D.D. reaction which may indicate a mucoprotein content.

Type B and Type C (Table 3) gland cells appear structurally identical and are in close proximity to each other. Their only apparent difference is in their chemical composition. These two gland types appear in the middle of the calcium spicules that support the rosette (Fig. 17). From this position the gland cells send necks up among the calcium spicules in the connective tissue (Fig. 19(until they reach the surface of the rosette (Fig. 18) where they pass to the surface via a secretory pore. The gland type given the arbitrary designation B gives a P.A.S. positive response indicating a possible neutral mucopolysaccharide, and the gland type designated as C contains an acid mucopolysaccharide.

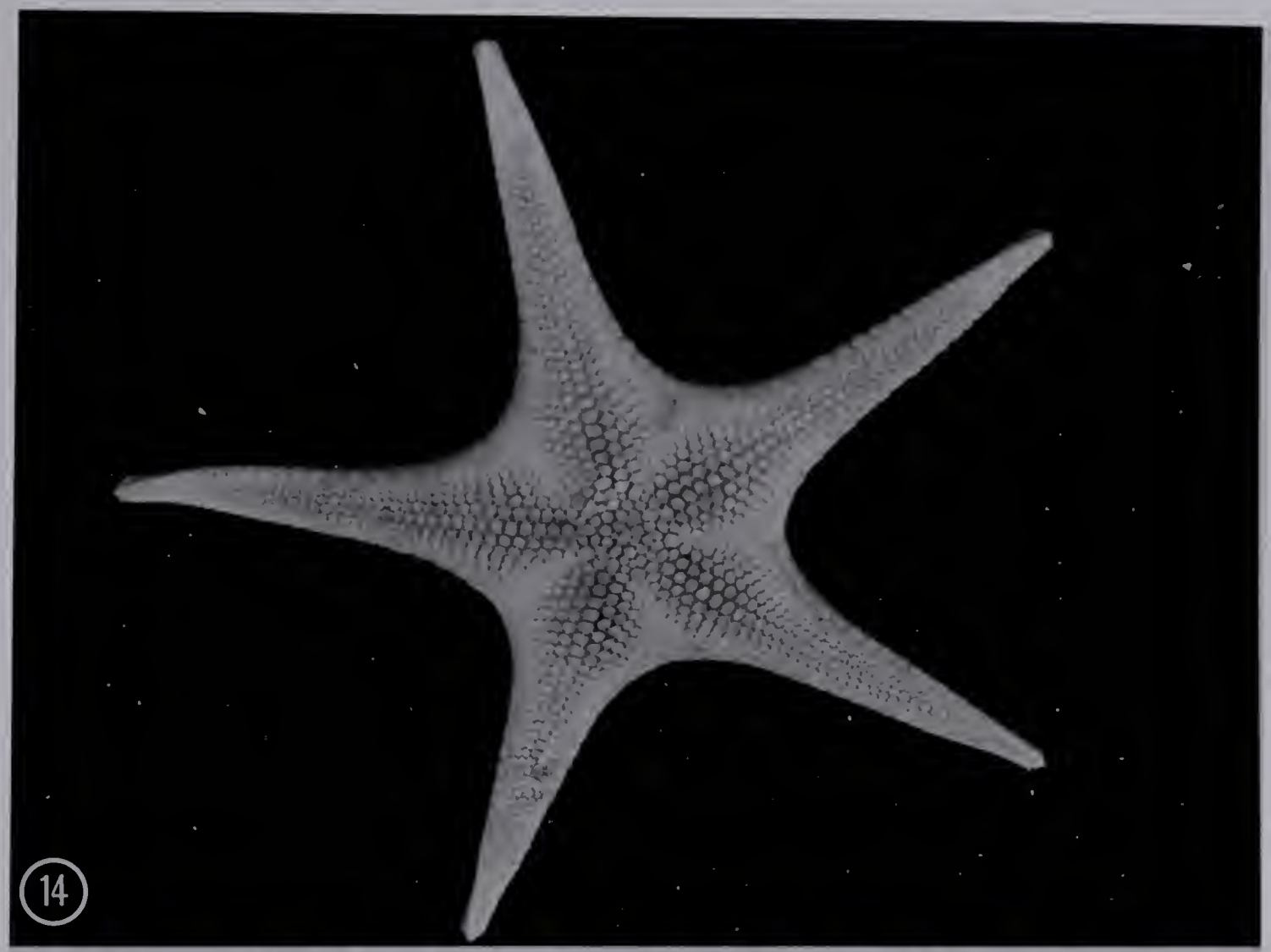
TABLE III

COMPARATIVE DATA ON THE ABORAL GLAND CELLS OF MEDIASTER AEQUALIS

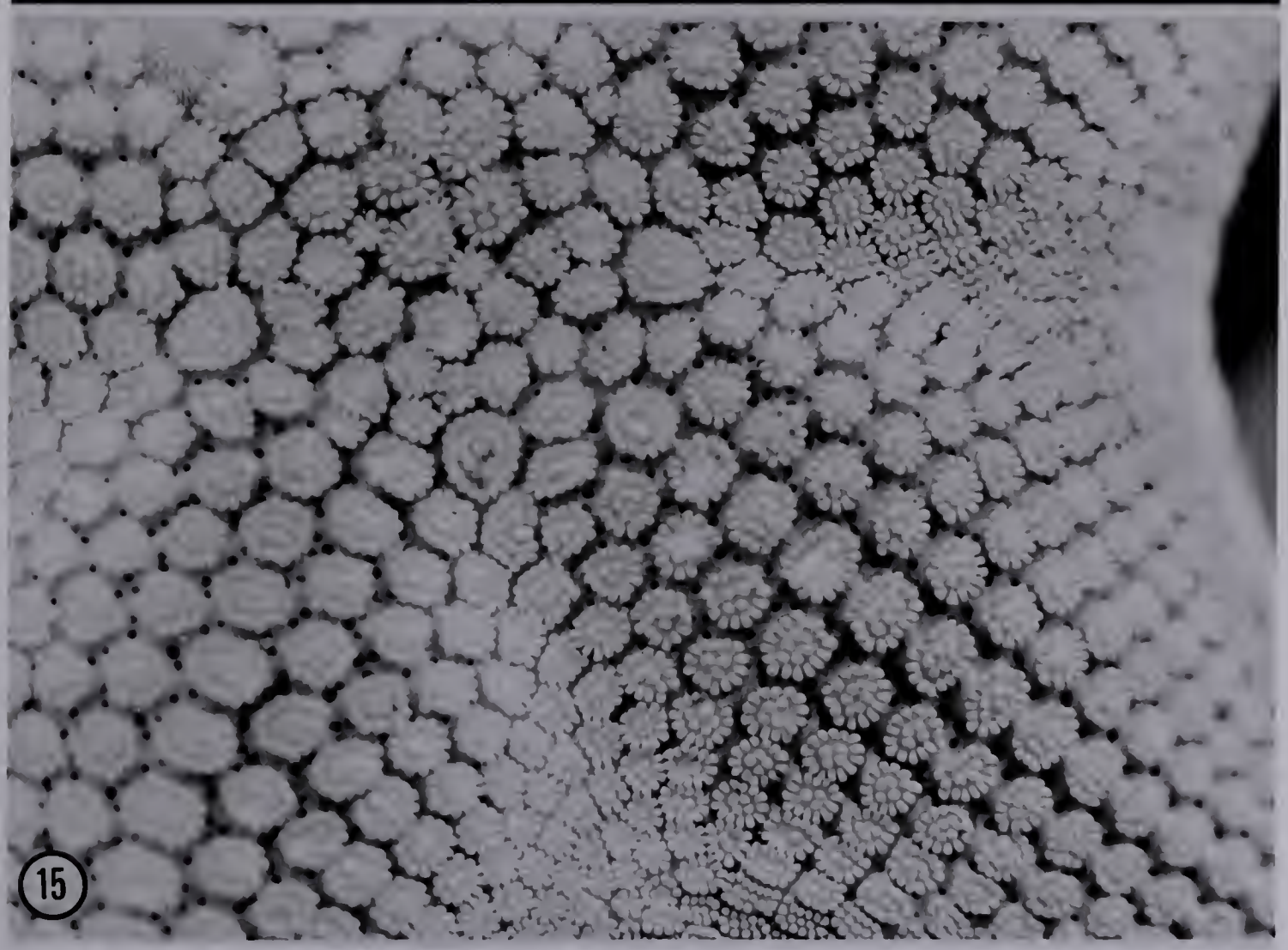
		Gland Type A	Gland Type B	Gland Type C
length		24 - 40 μ	77 μ	77 μ
width	neck	7 μ	4.5 μ	4.5 μ
	base	15 μ	9 μ	9 μ
secretory particle diameter		1.5 μ	1.5 μ	1.5 μ
chemical composition of gland cell		possible muco-protein	P.A.S. +	acid mucopoly-saccharide
opening to exterior		yes - pore	yes - pore	yes - pore
approximate ratio in epidermis		60%	20%	20%
location of nucleus		base	base?	base?
distribution in epidermis		edges of primary paxillae	inside secondary paxillae	inside secondary paxillae
Remarks				

Fig. 14. Aboral surface of Mediaster aequalis. Dried specimen. X1.

Fig. 15. Magnified area of the aboral surface of Mediaster aequalis showing the paxillary arrangement. X 15.



14



15

Fig. 16. Photomicrograph showing the gland cells found in the primary inter paxillary grooves of Mediaster aequalis. These gland cells occur in the epidermal tissue region only.

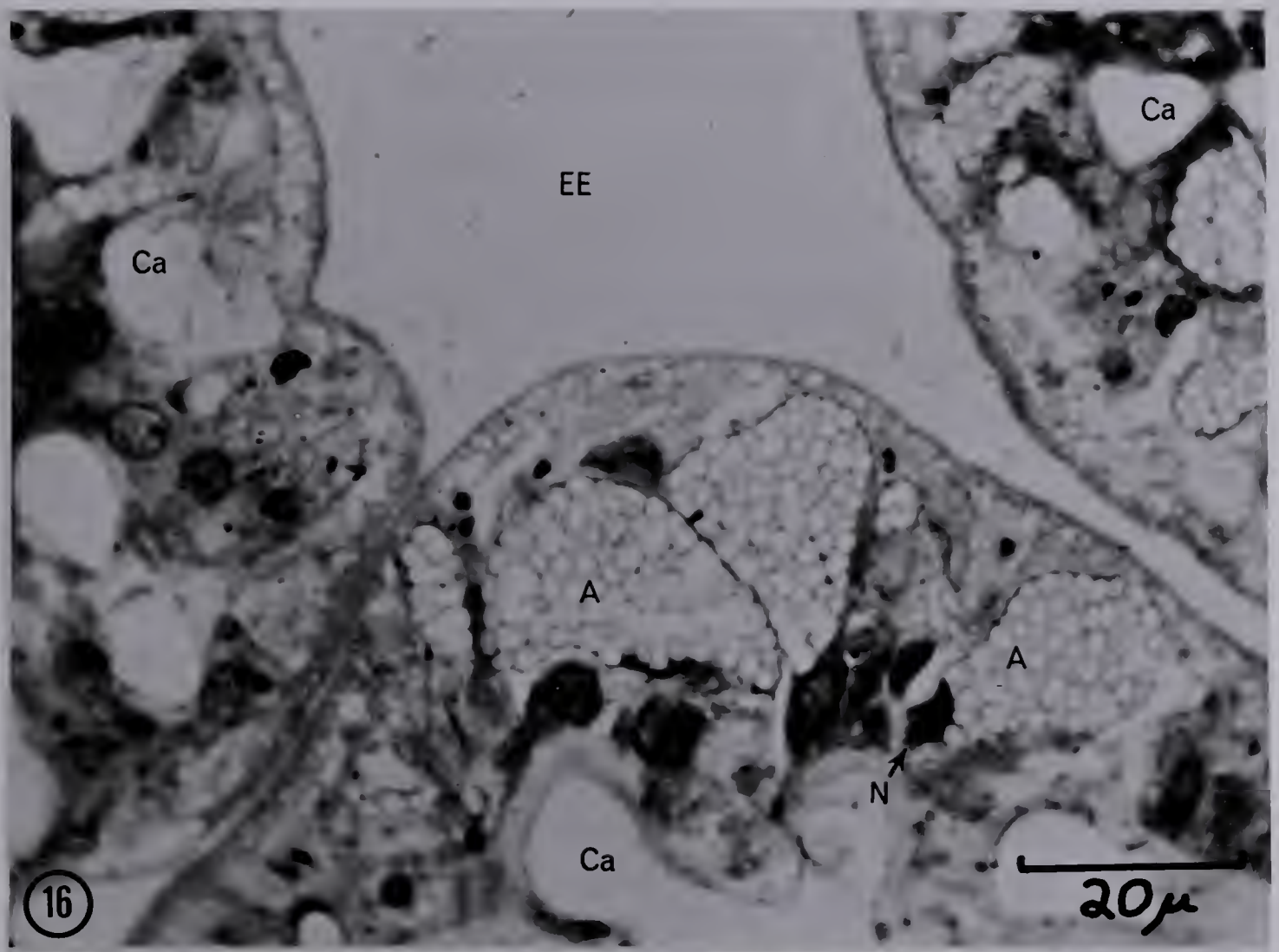
A, Type A gland cell; Ca, site of calcium spicule; EE, external environment; N, nucleus.

Bicarbonate/osmium fixation, Epon 812 embedding, Richardson's staining.

Fig. 17. Photomicrograph sh owing the gland cell bases which occur among calcium deposits at the base of the paxillae which cover the surface of Mediaster aequalis.

BC, B or C type gland cell; Ca, site of calcium deposit; N, nucleus.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's staining.


Ca
EE
Ca
Ca
A
A
N
Ca
16
20μ

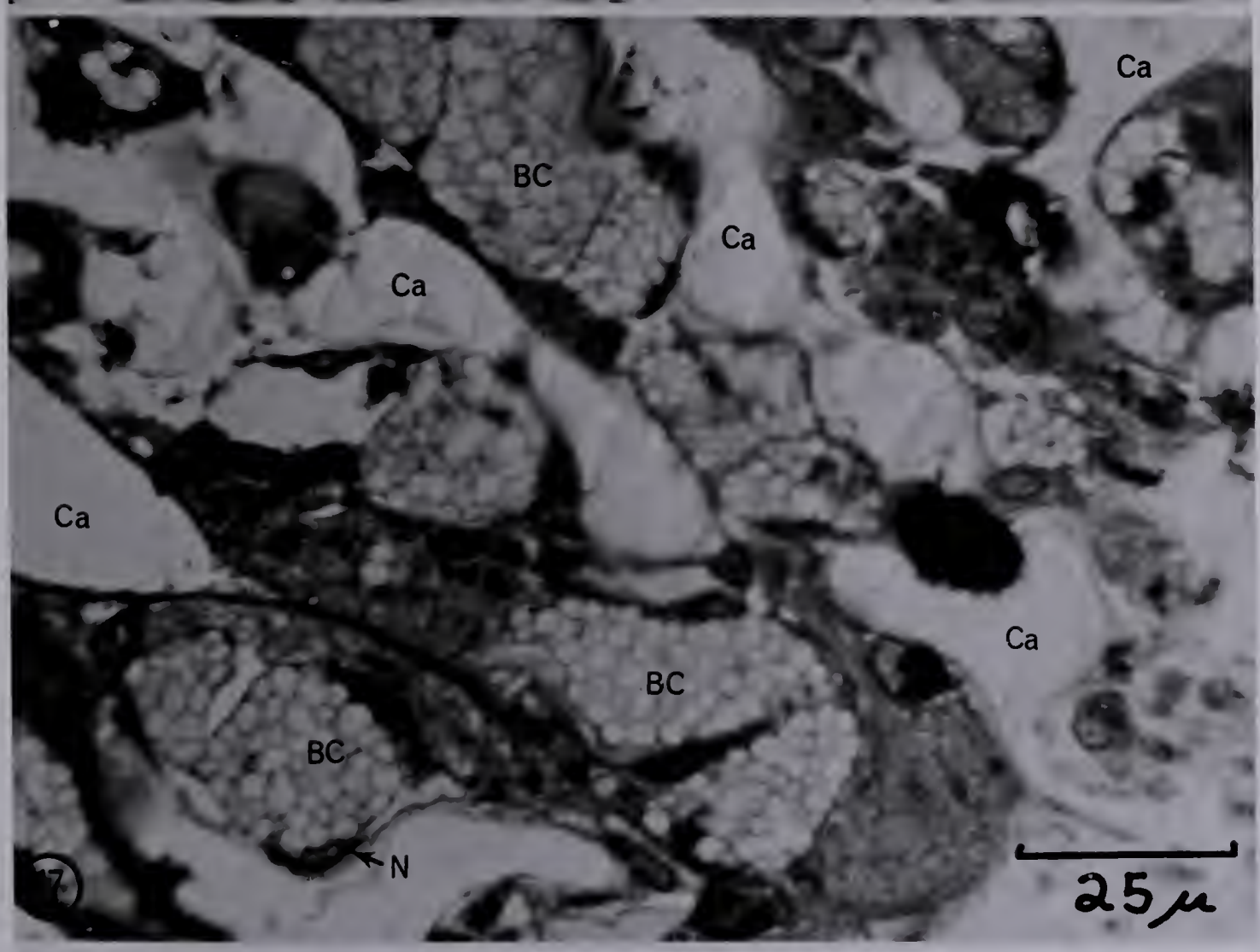

Ca
BC
Ca
Ca
Ca
Ca
BC
BC
N
25μ

Fig. 18. Cross section through the surface of a secondary paxilla on Mediaster aequalis showing the gland from deep inside the paxilla about to open to the surface.

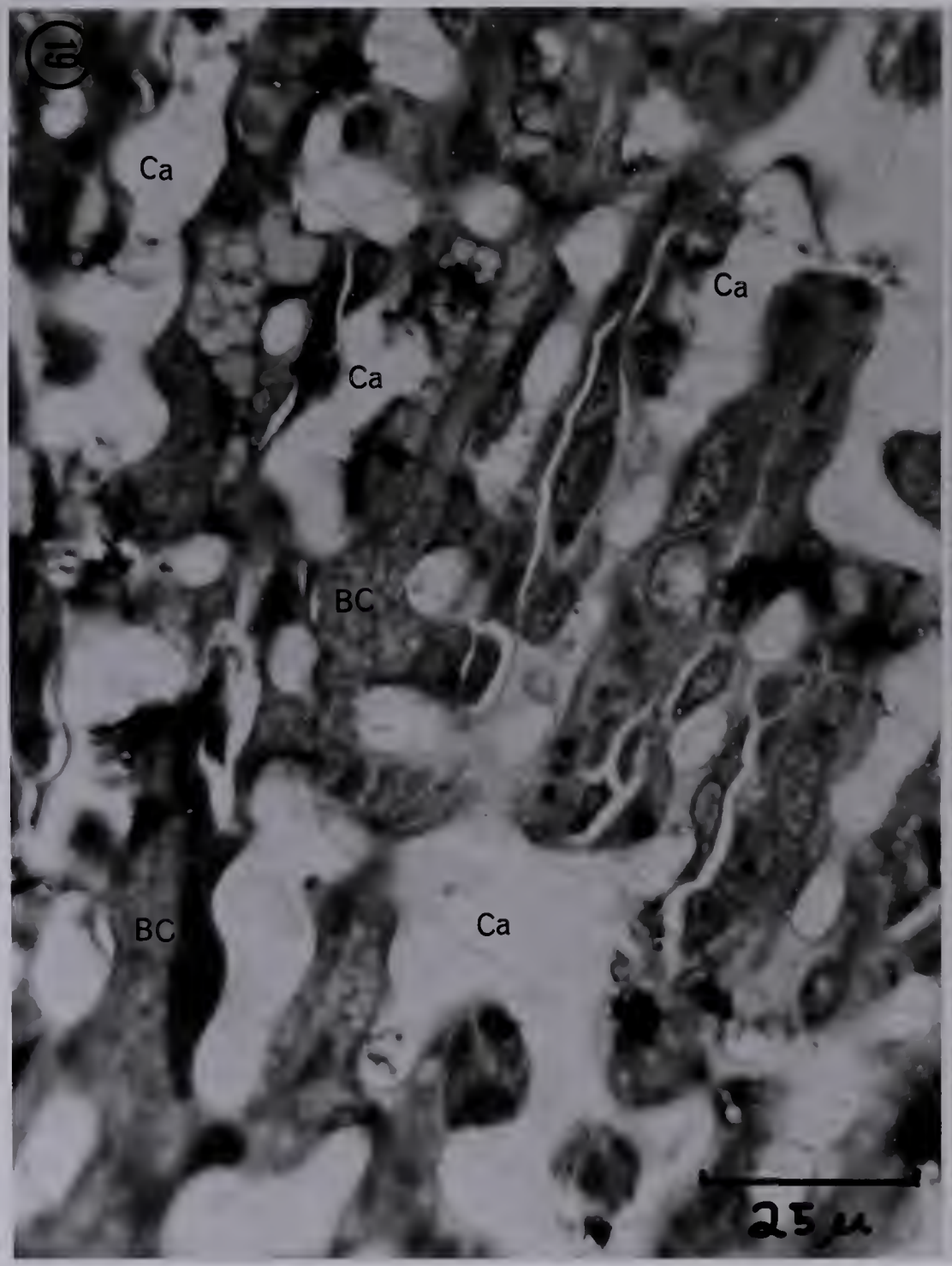
BC, B or C type gland cell; Ca, site of calcium deposit; EE, external environment.

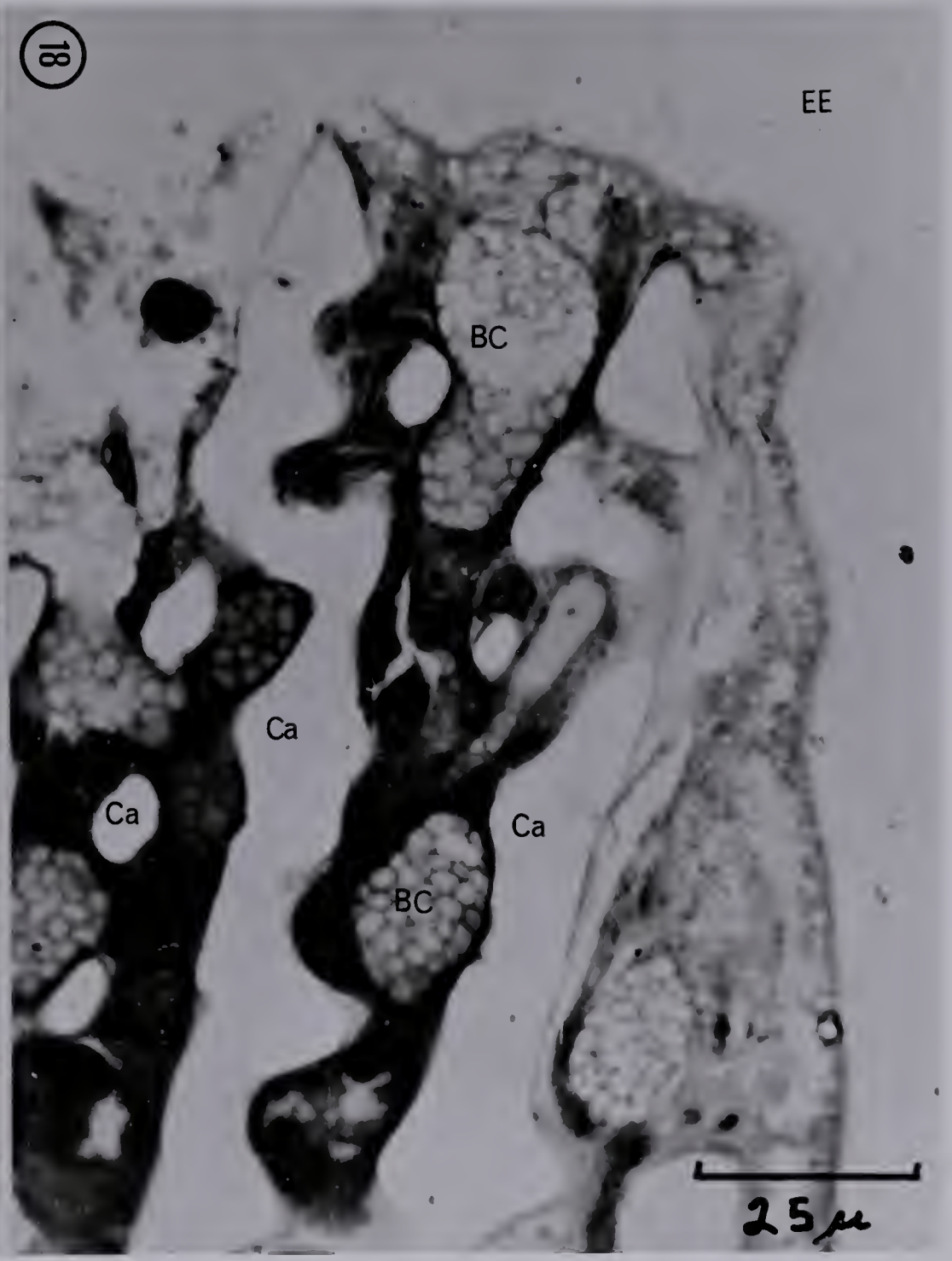
Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson,s staining.

Fig. 19. Photomicrograph showing the gland cell necks as they move up between the heavy calcium deposits which occur in the paxilla of Mediaster aequalis.

BC, B or C type gland cell; Ca, site of calcium deposit.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's staining.


19
Ca
Ca
Ca
BC
BC
Ca
25μ


18
EE
BC
Ca
Ca
Ca
BC
25μ

D. Hippasteria spinosa

The epidermis of H. spinosa is composed of ciliated columnar epithelium, exhibiting a uniformly cuticular appearance 2.8μ deep on its dorsal surface, and which forms a continuous epidermal layer of varying depth over the entire surface of the animal. This epidermal layer impinges directly on the calcareous deposits and fenestrations of the endoskeleton. No definite structure resembling a basement membrane could be resolved with light microscope (Fig. 22). The cytoplasm above the nuclei appeared to be highly vacuolated (Fig. 24, 25). In certain areas of the epidermis deep invaginations were observed which probably aid this highly calcified animal in flexing.

At least three types of gland cell have been recognized in H. spinosa. A fourth type of tissue has been observed, the glandular character of which could not be determined with certainty.

Cells of Type A (Fig. 22) (Table 4) are found throughout the aboral surface, primarily on the large spines (Pages 23,D). The body of this gland is found immediately below the main epidermal layer and occurs between the calcium spicules of the endoskeleton. These structures have a large nucleated base with a long thin neck leading to the surface pore. Glandular cells of Type A occurred in clusters of from two to four cells. Histochemical tests show that this type of gland contains acid mucopolysaccharide (Fig. 23).

Gland cells of Type B (Table 4) have a distinctive structure but are rare in the epidermis. Precise distribution can only be shown by further study. This type of gland cell appeared to have its nucleus close to the base with distinct secretory packets occupying the upper part of the cell body. The secretory packets are different than those found in other asteroids studied in that they take the form of spheres which have a dense or thick wall (Fig 24). The gland itself was very narrow, extending the length of the epidermis in which it occurred and opened to the exterior via a secretory pore. Histochemical tests did not give a positive response to any structure of this type but as I was not able to locate a specific area where these gland cells occurred with any degree of repetition, specific histochemical results could not be obtained.

A third type of gland cell (Type C) was found in H. spinosa (Table 4). These cells have unique structural features, and were one of the most unusual found in this study. They occur only in specific areas of the aboral epidermis, but in this case it has been possible to calculate their position, with relation to external epidermal structures. Their secretory pores open to the surface in the most depressed areas of the surface epidermis, between the smallest of the surface protuberances (Fig. 21, no. 2) and never on any of the paxillae or pedicellariae. In this area the calcium endoskeleton extends from the base of the epidermis to deep into the body of the

animal with no breaks for the passage of muscles or dermal branchiae as occur in other areas. It is throughout the fenestrations between the solid calcium deposits in the endoskeleton that gland cells of Type C are found. The gland itself is extremely long (1000μ) in comparison with gland cells previously described. In passing from the base to the surface it follows a tortuous course between the calcium deposits in the endoskeleton, making it difficult to follow the progress of a single cell. The base of the cell does not contain distinct secretory packets as has been found in other asteroid cells, but instead appears to contain a substance which has a lace-like form (Fig. 29). This internal structure could be a fixation artifact in spite of the fact that other components appear to be well fixed. Moving toward the surface, this initial internal form becomes more compact (Fig. 28). In approximately the middle of the gland distinct secretory packets were observed (Fig. 26, Fig. 27). This internal composition is retained as the cell neck passes up through the epidermis (Fig. 24) and exists at a secretory pore. These gland cells never occur alone but always in distinct clusters.

The histochemistry of these glands was puzzling. They showed a slight positive reaction to both tests for acid mucopolysaccharide and also the P.A.S. reaction, however neither was strong enough to be a main component of the gland cell. Sudan Black B showed a strong positive reaction when used with

Paraplast embedded sections. No reaction with Sudan Black B is observed in gelatin embedded sections. This was not done in conjunction with lipid extraction, so no further conclusions other than the possibility of some lipid type could be considered.

The possibility that H. spinosa possesses a fourth type of glandular cell is based upon the fact that certain cells of an apparent glandular nature are visible in parts of the epidermis when fixed in Baker's formal-calcium, decalcified with EDTA, sectioned with a freezing microtome and immersed in Oil Red O or Sudan Black B. The area incorporating the coloring material occurs in the top half of the epidermal layer; sections cut from Epon 812 embedded material have not yet shown a structure resembling this type of cell. While the histochemistry tends to suggest the possibilities of a lipid gland, it would be premature to include these cells in the list of gland cells of H. spinosa until more information is available.

TABLE IV

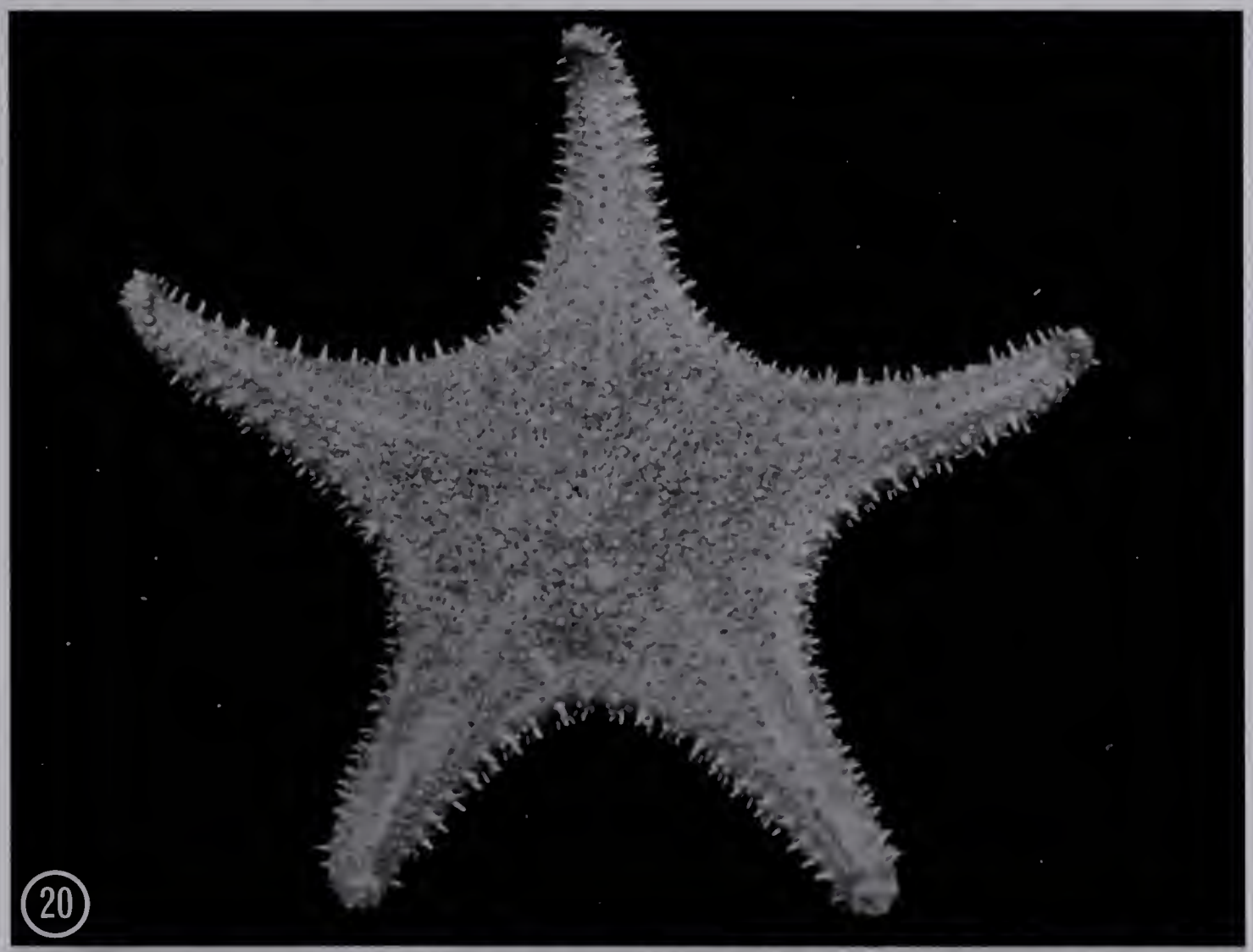
COMPARATIVE DATA ON THE ABORAL GLAND CELLS OF HIPPASTERIA SPINOSA

		Gland Type A	Gland Type B	Gland Type C
length		210 μ	50 - 100 μ	1000 μ
width	neck	1.2 μ	3 - 5 μ	7.2 μ
	base	1.4 μ	3 - 5 μ	2.5 μ?
secretory particle diameter		1.2 μ	1 μ	1 μ
chemical composition of gland cell		acid mucopoly-saccharide	unknown	lipid?
opening to exterior		yes - pore	yes - pore	yes - pore
approximate ratio in epidermis		70%	30%	--
location of nucleus		base	base	unknown
distribution in epidermis		primarily on spines	on protuberances	not in epidermis
Remarks				

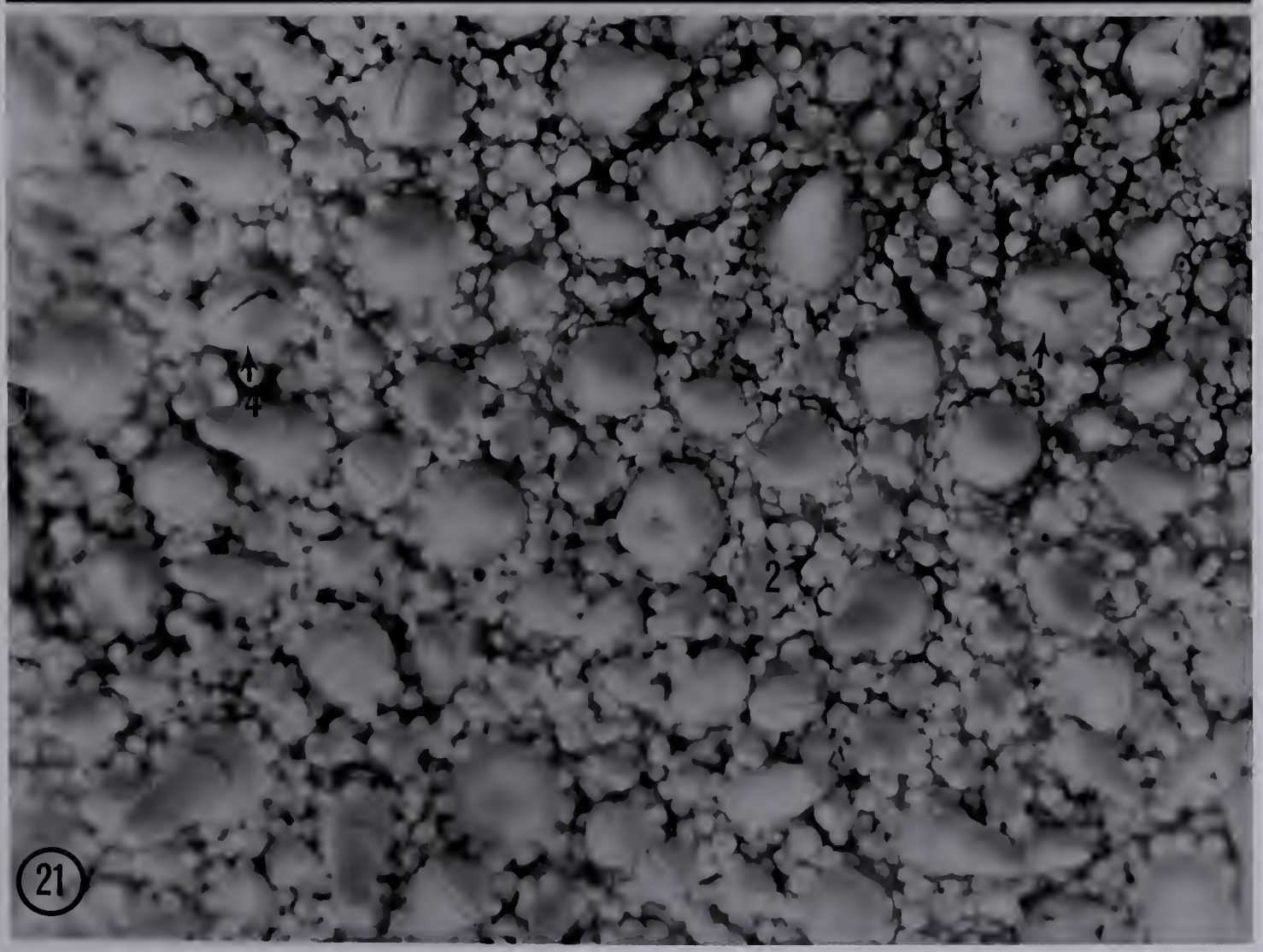
Fig. 20. Aboral surface of Hippasteria spinosa. Dried specimen. X ½.

Fig. 21. Details of the aboral epidermal structures of Hippasteria spinosa. Dried specimen. X 4.

1, large spines; 2, small protuberances; 3, trivalved pedicellariae, 4, bivalved pedicellariae.



20



21

Fig. 22. Cross section of the epidermal covering on the large spines (Fig. 21, 1) of Hippasteria spinosa.

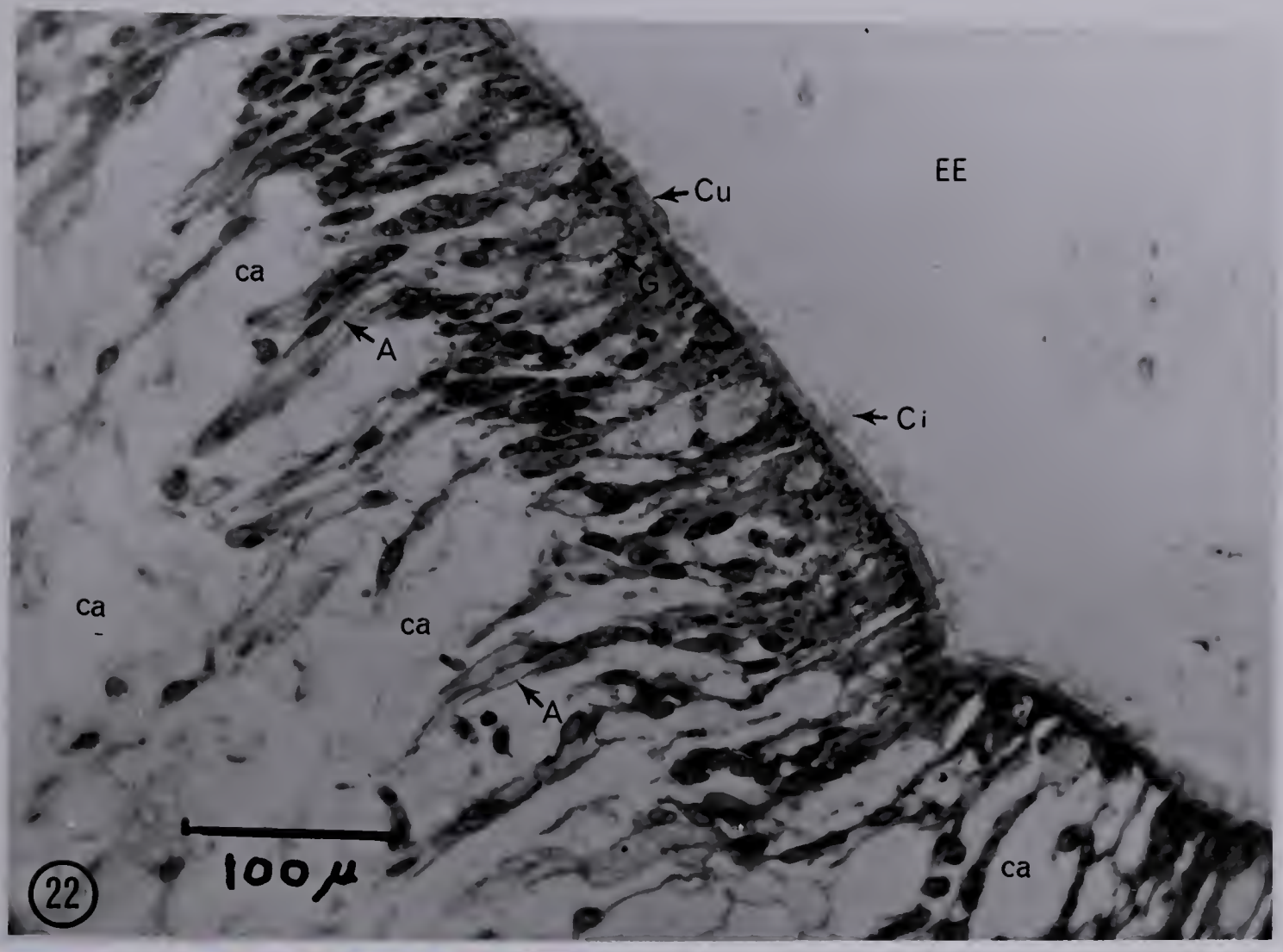
A, Type A gland cell; Ca, site of calcium spicule; Ci, cilia, Cu, cuticle; EE, external environment; G, unidentified gland cell.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's staining.

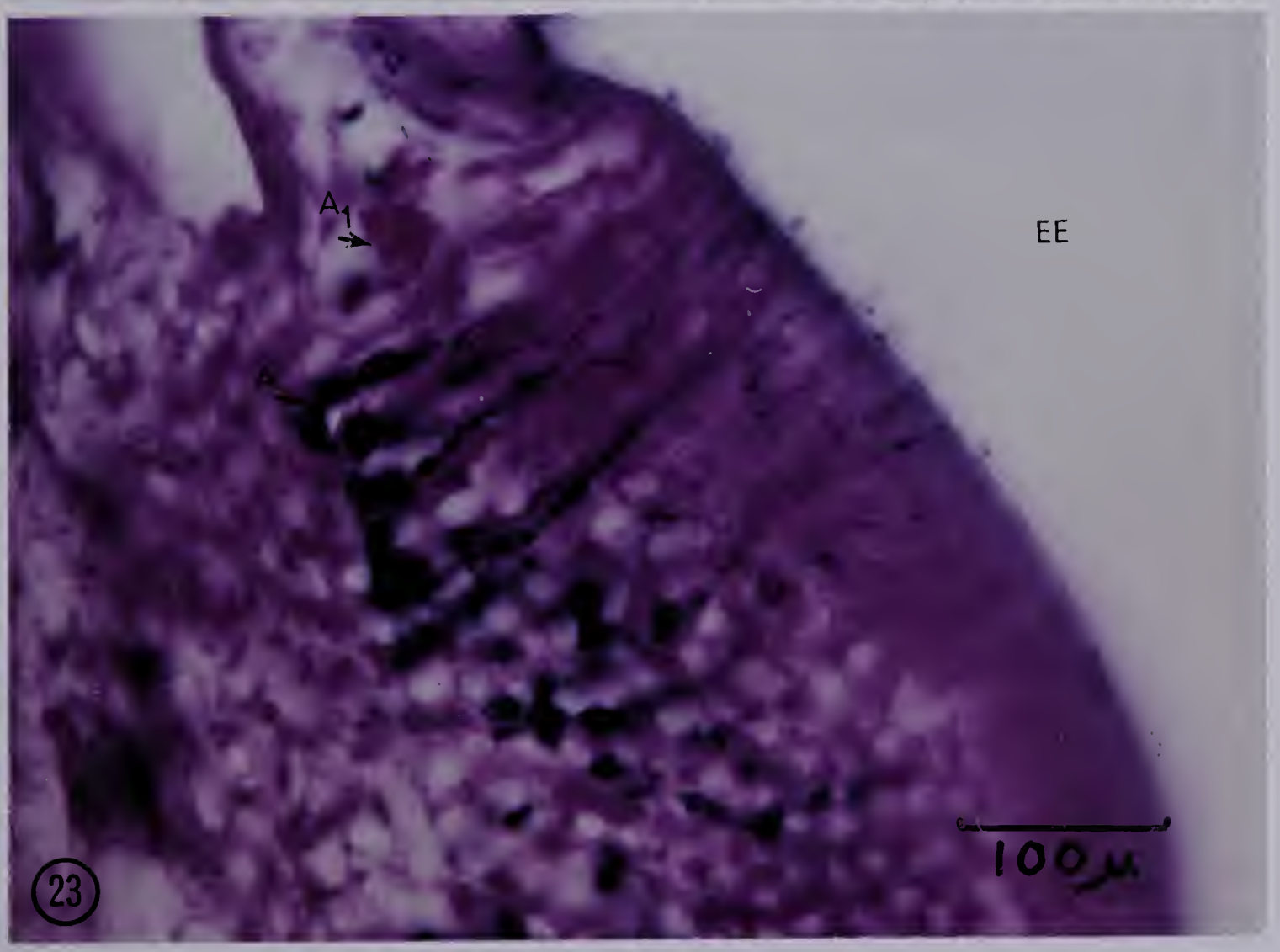
Fig. 23. Histochemical staining of the same area as shown above (Fig. 22) indicating the presence of two gland types which have different chemical components.

A, Gland cell showing Alcian Blue positive response; A , gland cell showing P.A.S. positive response, EE, external environment.

SUSA fixation, Paraplast embedding, and P.A.S.-Alcian blue staining.



22



23

Fig. 24. Cross section through the epidermis near the small protuberances (Fig. 21, 2) on the aboral surface of Hippasteria spinosa.

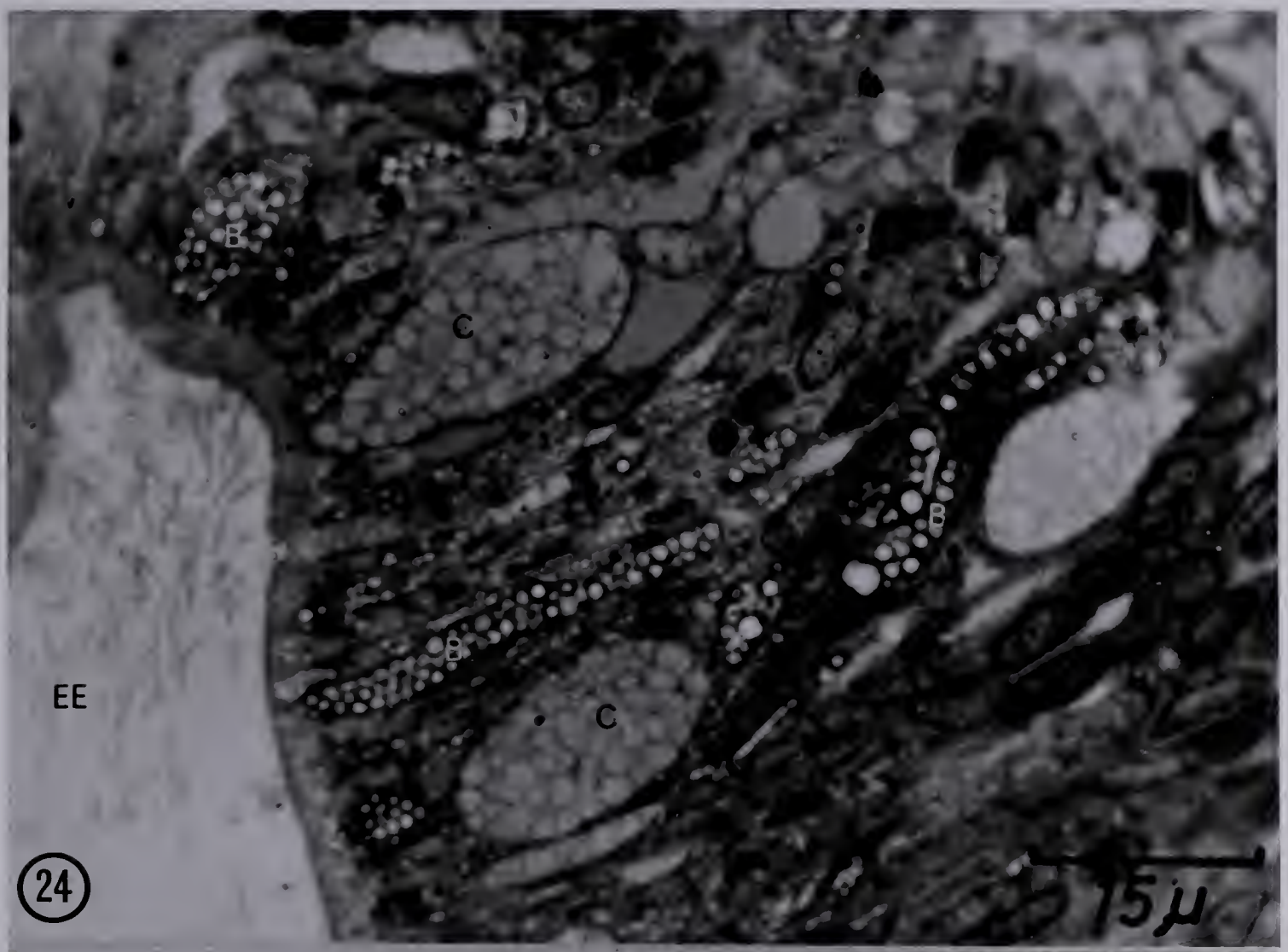
B, Type B gland cell; C, Type C gland cell; EE, external environment.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's staining.

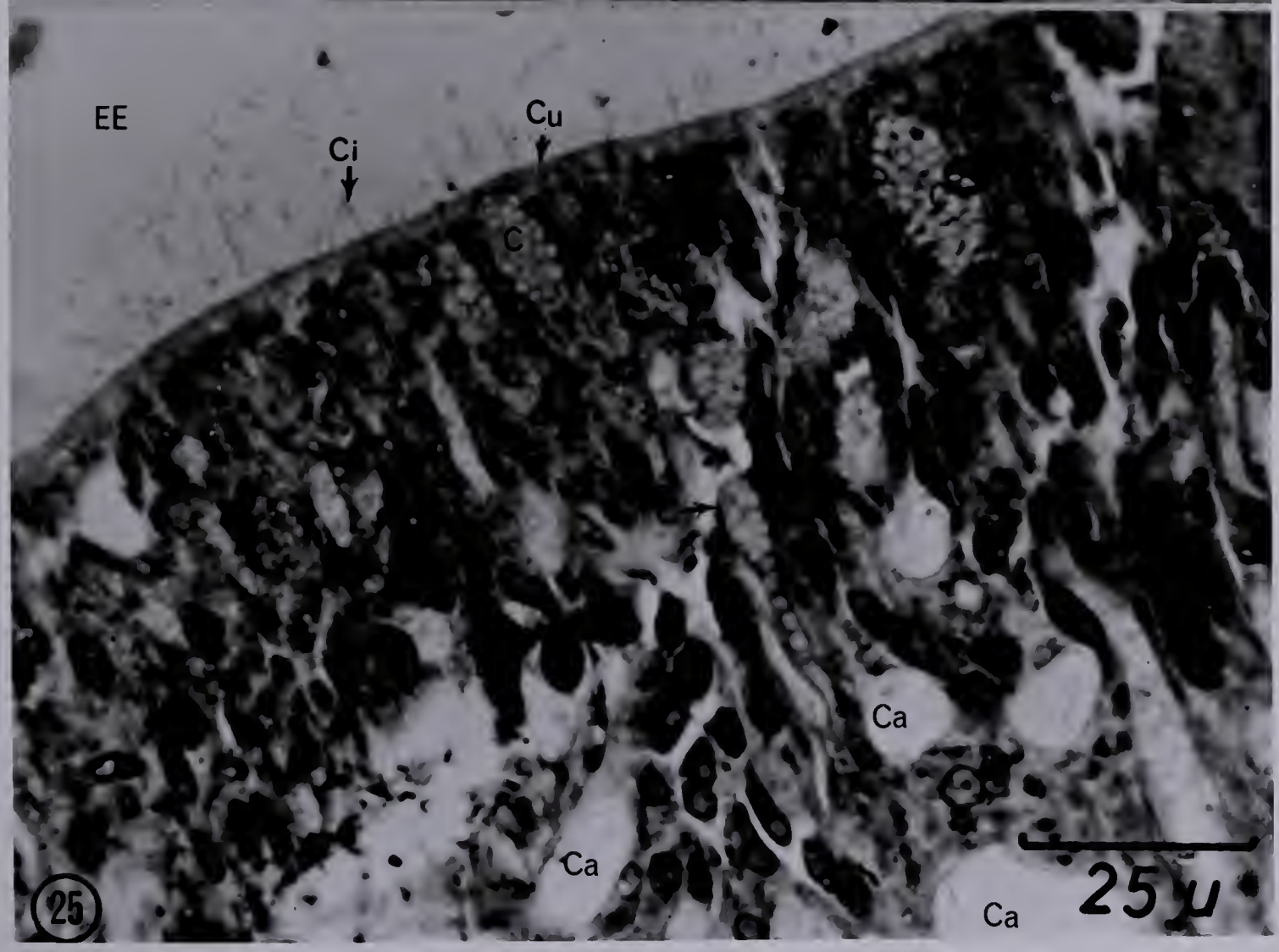
Fig. 25. Cross section of the epidermal tissue occurring between the small protuberances (Fig. 21, 2) on the aboral surfaces of Hippasteria spinosa. Gland Type C is only found in certain sections of the animal, but where it does occur it always secretes to the environment from this portion of the epidermis.

C, top part of Type C gland cell; Ca, site of calcium spicule; Ci, cilia; Cu, cuticle; EE, external environment.

Bicarbonate/osmium fixation, Epon 812 embedding, Richardson's staining.



(24)



(25)

Fig. 26. Photomicrograph showing continuation of the Type C gland cells from Fig. 24 into the heavily calcified area directly under the epidermis. This area occurs under the epidermal region shown in Fig. 25.

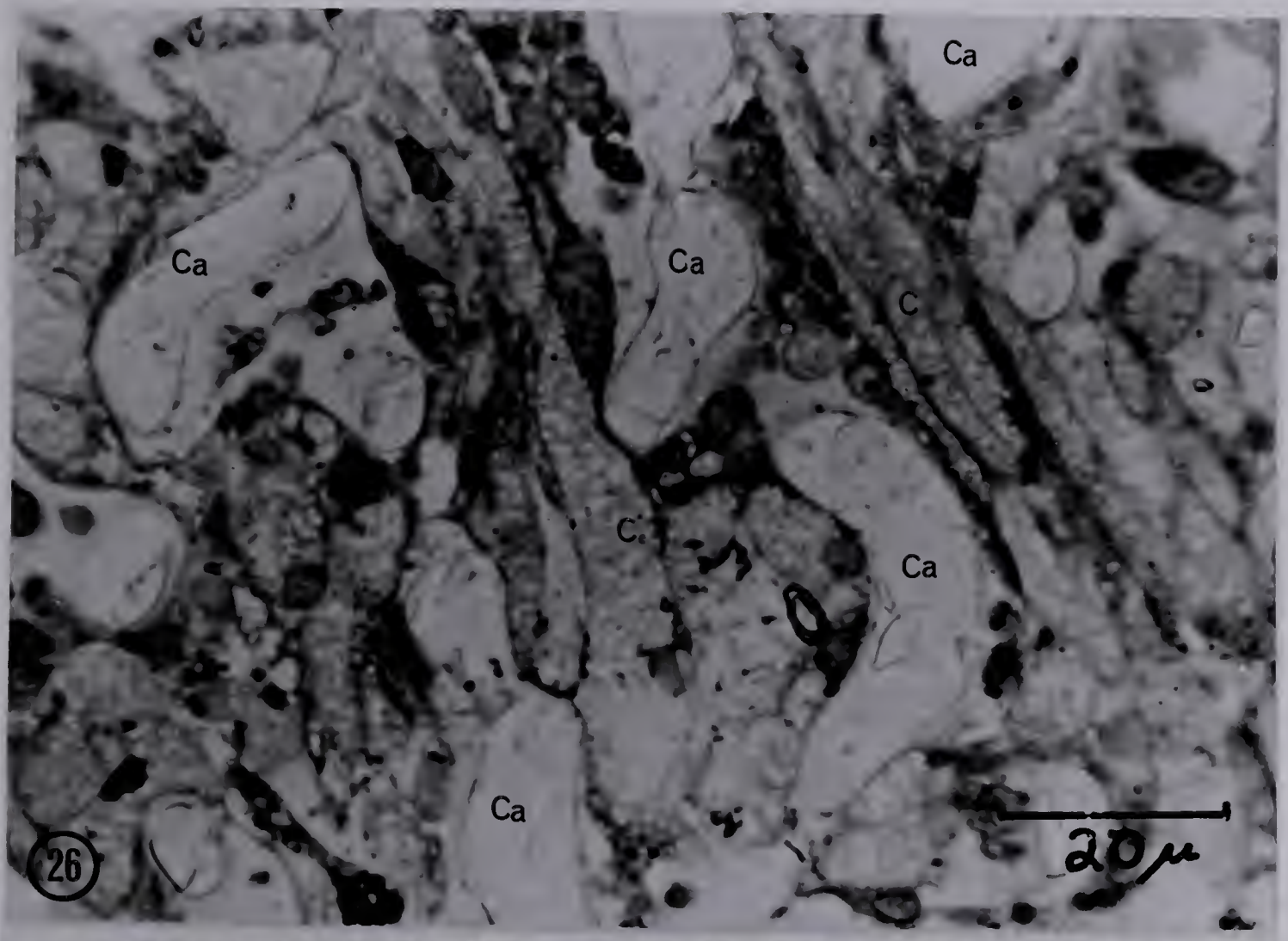
C, Type C gland cell; Ca, site of calcium spicules.

Bicarbonate/osmium fixation, Epon 812 embedding, Richardson's staining.

Fig. 27. The same area as the above (Fig.26), but at a higher magnification to show more detail in the Type C gland cell.

C, Type C gland cell; Ca, site of calcium spicule; N, nucleus.

Bicarbonate/osmium fixation, Epon 812 embedding, Richardson's staining.


Ca
Ca
Ca
C
C
Ca
Ca
26
20μ

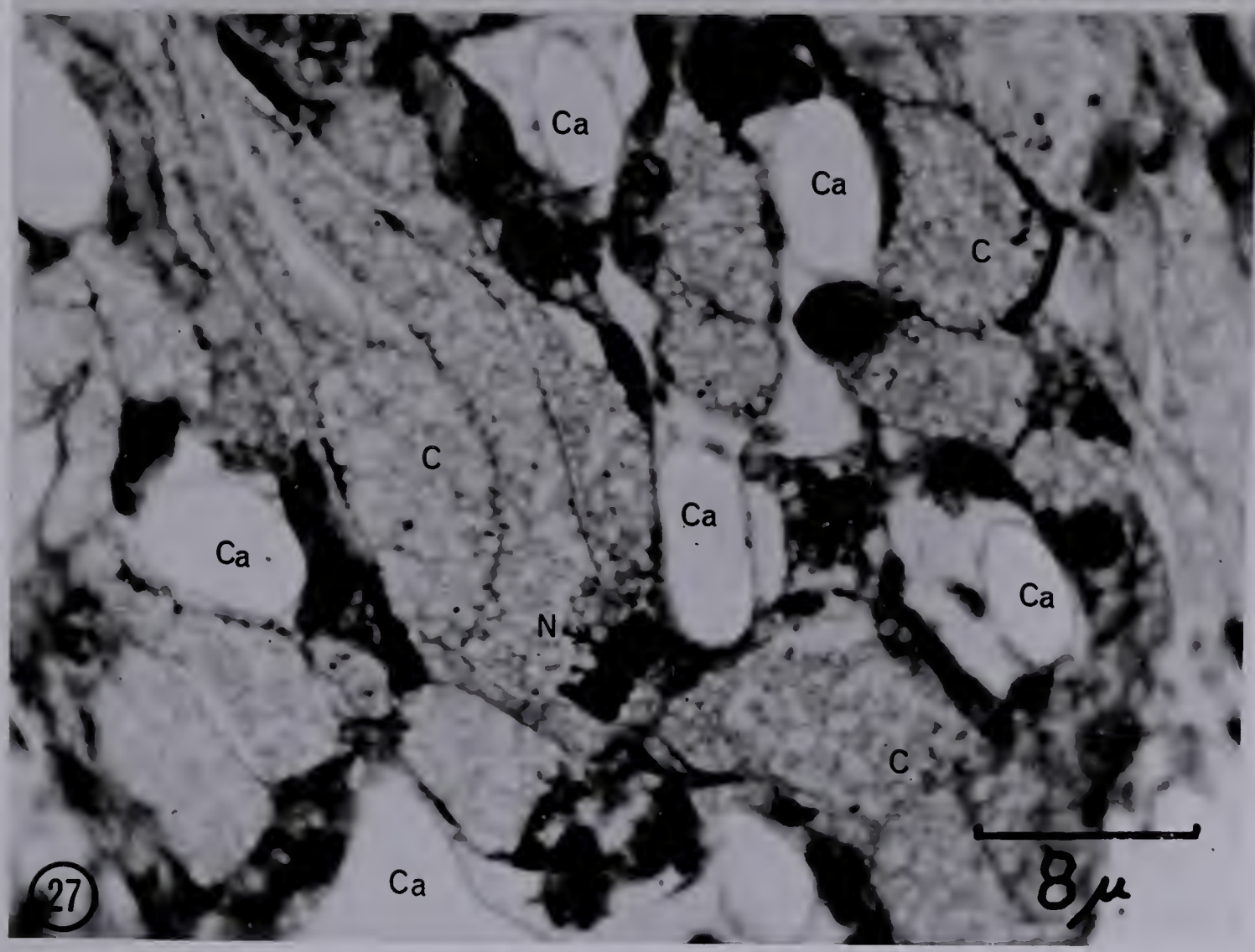

Ca
Ca
C
C
Ca
Ca
N
Ca
C
Ca
27
8μ

Fig. 28. Type C gland cells occurring approximately 700 u from the surface.

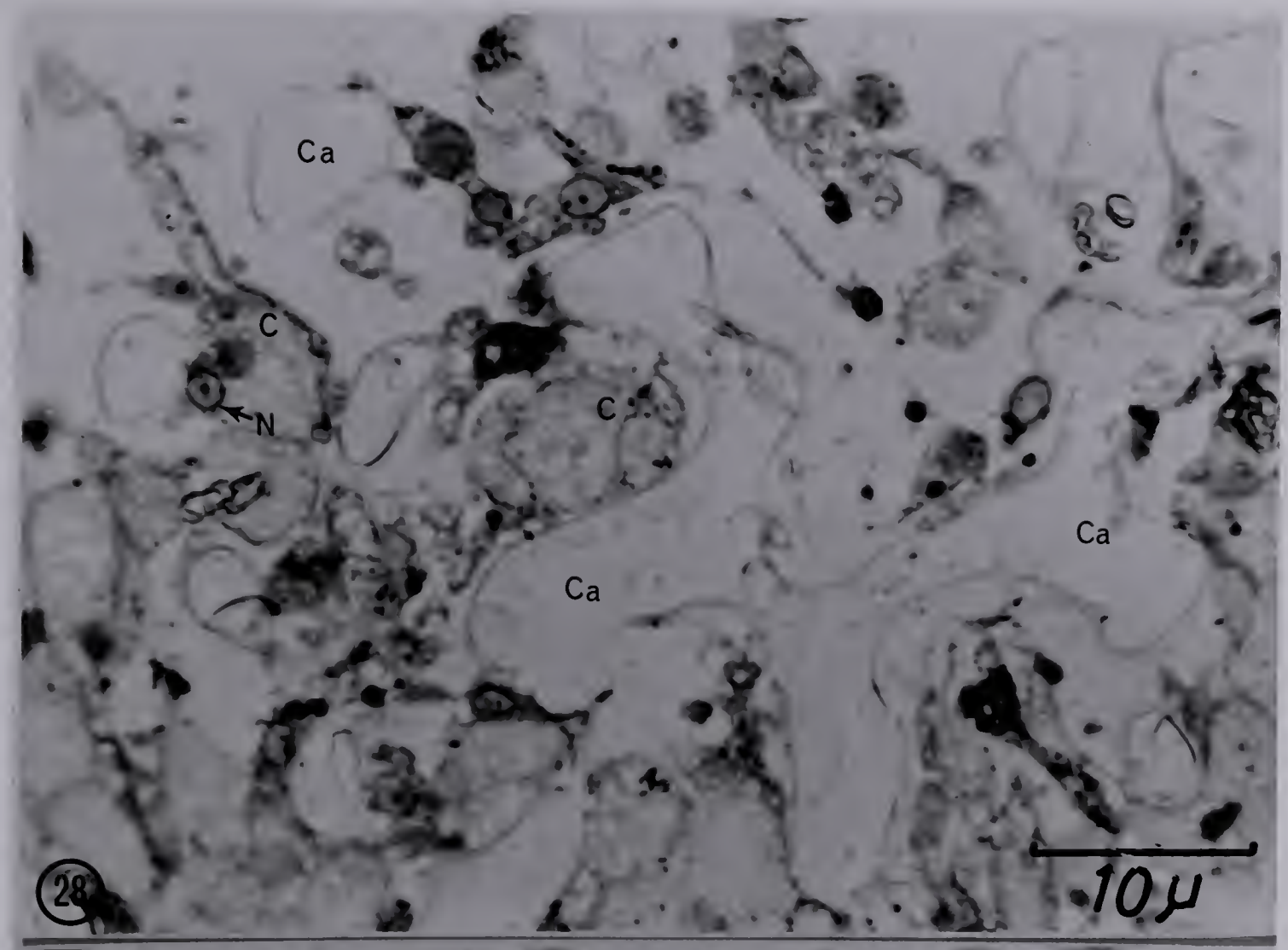
C, Type C gland cell bodies; Ca, site of calcium spicules.

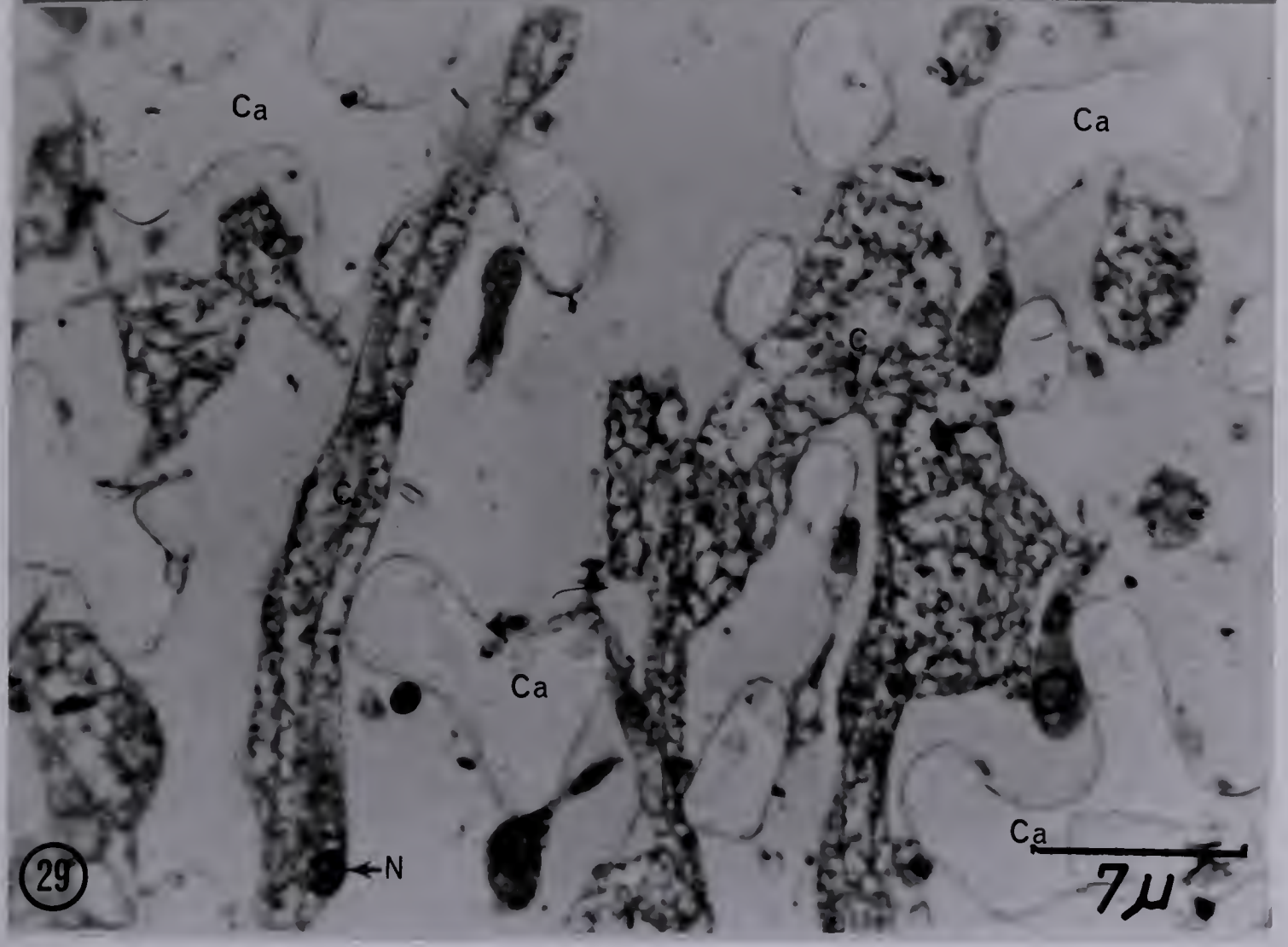
Bicarbonate/osmium fixation, Epon 812 embedding, Richardson's staining.

Fig. 29. The typical structure of the Type C gland cell at its base, approximately 1000 u from the surface.

C, Type C gland cell, Ca, site of calcium spicule, N, nucleus.

Bicarbonate/osmium fixation, Epon 812 embedding, Richardson's staining.


Ca
C
N
C
Ca
Ca
28
10μ


Ca
Ca
C
C
Ca
Ca
N
29
7μ

E. Pteraster tesselatus

On the aboral surface of the cushion star, (Fig. 30) occurs a supradorsal membrane supported by paxillae. This structure covers the nidamental chamber, the floor of which composes the true epidermis of the animal (Rodenhouse & Guberlet 1946) (Fig. 31, Fig. 32). The thick supradorsal membrane is covered by an epidermal layer composed of stubby, columnar cells resting on a basement membrane which distinctly sets this tissue apart from the underlying supradorsal membrane. This surface epidermal layer has gland cells (Type A) which appear to be interspersed at random between the epidermal cells (Table 5) (Fig.33). These gland cells contain nuclei in their lower halves and are completely filled with secretory packets which appear to be released to the environment by a rupturing of the surface membrane of the cell. Histochemical tests show the Type A gland cells to contain both acid mucopolysaccharide and P.A.S. positive material, probably neutral mucopolysaccharide.

Passing through the supradorsal membrane are numerous canals which eventually lead to the nidamental chamber (Fig.32) (Rodenhouse & Guberlet 1946). Each of these canals lead into a gland sac (Fig. 32, 34). All of the sacs and their canals compose the main gland complex of the supradorsal membrane. As the main canal from the surface of the supradorsal membrane passes through the gland sac, smaller secondary canals from the gland complex itself feed into the main canal (Fig. 32).

Surrounding these secondary canals are the secretory gland cells proper which comprise the basic glandular unit of the main gland. (Fig. 37). These secretory gland cells are found in two different types. The gland cell which is the most common, is designated a gland unit (Fig. 37) and appears as a sac-like container, filled with large secretory packets. These are evidently released into the secretory canal by the rupture of the top of the gland cell where it is exposed to the canal. Neither nuclei nor cytoplasmic material have been observed in this structure. Histochemical tests show these cells to contain both acid mucopolysaccharide and a P.A.S. positive material which is possibly a neutral mucopolysaccharide. The second type of gland cell found in the gland sacs is Type B (Table 5). This cell occurs much less frequently than gland units but in the same position (Fig. 38). The structure of the secretory packets is unusual in that a dense sphere occurs in the centre. These cells, as in the gland units appear to release their contents by rupture into the secretory canal. Histochemical tests show these gland cells have positive reaction for protein and a positive indication for lipid.

The last two gland types which have been found on the aboral surface of this animal, Types C and D, compose the true epidermis of P. tesselatus which is found on the floor of the nidamental chamber and on the sides of the paxillae which support the supradorsal membrane. There may

be a continuum between this layer and the main gland complex of the supradorsal membrane, however this has not yet been explored. Gland cells of Type C (Table 5) contain large secretory vesicles which fill the entire gland, and which were released by the rupture of the cell into the nidamental cavity (Fig. 39). The gland appears to have a nucleus in its lower part, with very little cytoplasm in the cell. This gland cell contains acid mucopolysaccharide (Fig. 40) and a P.A.S. positive component which is possibly a neutral mucopolysaccharide. The second gland type which occurs in this area resembles the previous Type C gland cell. It occurs with the Type C cell in the true epidermis of P. tesselatus and was called Type D. Type D gland cell (Table 5) has dense secretory packets which are slightly smaller than Type C, (Fig. 39) but otherwise resembles it very closely. Histochemically the only positive reaction which was obtained were the D.D.D. and ninhyrin tests indicating the presence of protein in the Type E gland cells (Fig. 41).

TABLE V

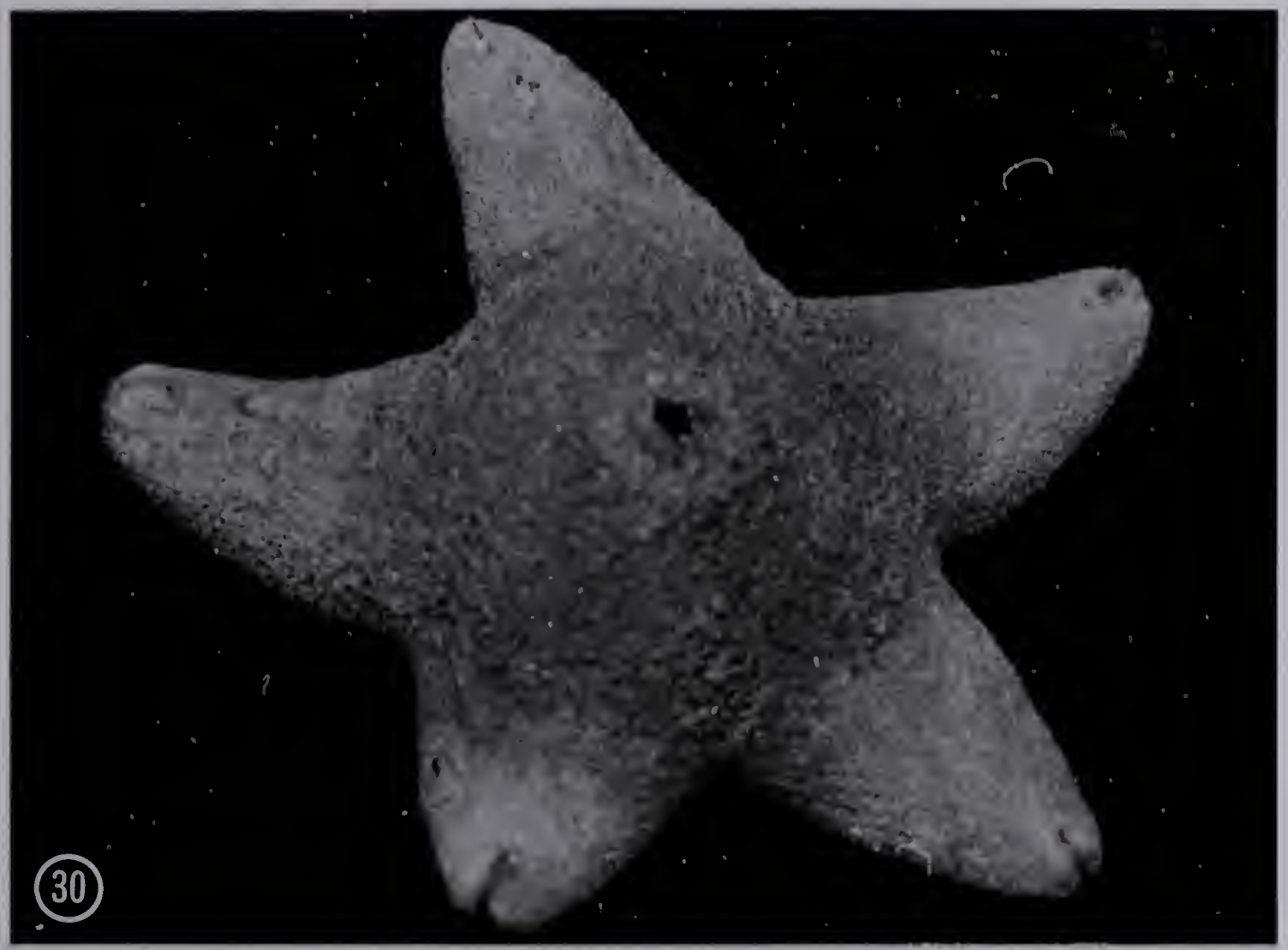
COMPARATIVE DATA ON THE ABORAL GLAND CELLS OF PTERASTER TESSELATUS

	Gland Type A Gland Unit Cell	Gland Type B	Gland Type C	Gland Type D
length	14 μ	?	?	60 μ	60 μ
width base	7 - 14 μ	20 μ	13 μ	28 μ	28 μ
width neck	7 - 14 μ	20 μ	13 μ	28 μ	28 μ
secretory particle diameter	0.8 μ	6 μ	1 μ	10 μ	3 - 5 μ
chemical composition of gland cell	acid muco-polysaccharide and P.A.S. positive	acid muco-polysaccharide and P.A.S. positive	protein and possible lipid	acid muco-polysaccharide and P.A.S. positive	protein
opening to exterior	yes, rupture	yes, rupture	yes, rupture	yes, rupture	yes, rupture
approximate ratio in epidermis	10%	--	--	50%	50%
location of nucleus	base	?	?	?	?
distribution in epidermis	entire	bottom half of supradorsal membrane	bottom half of supradorsal membrane	true epidermis on floor of nidimental chamber	true epidermis on floor of nidimental chamber
Remarks	on epidermis of supradorsal membrane				

Fig. 30. Aboral surface of the cushion star, Pteraster tesselatus. Dried specimen. X ½

Fig. 31. Cross section of the entire aboral surface of Pteraster tesselatus. (Refer to Fig. 32). Dried specimen. X ½.

EE, external environment; P. paxilla supporting supradorsal membrane.


30

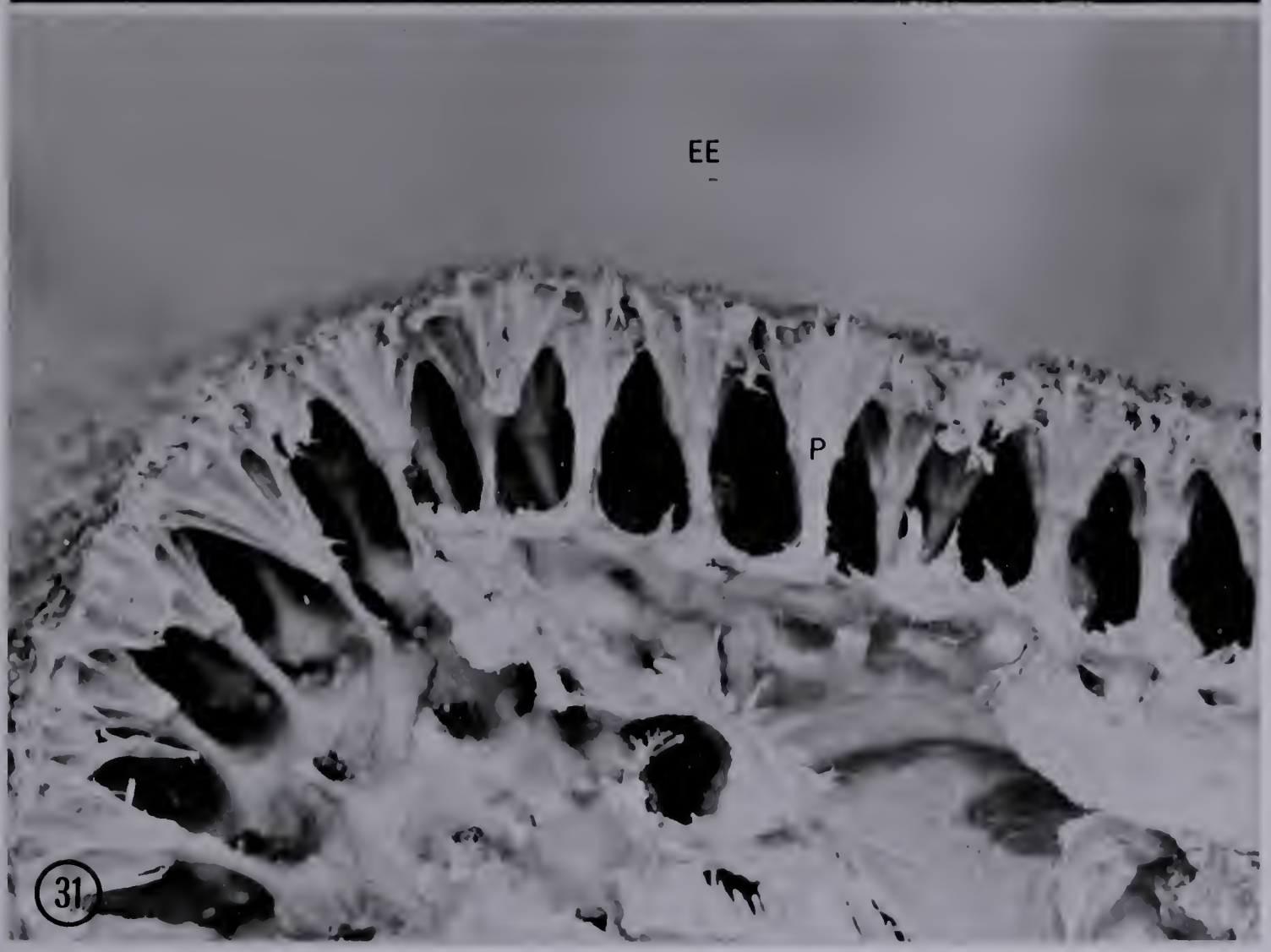

EE
P
31

Fig. 32. A diagrammatic representation of the aboral surface (supradorsal membrane and true epidermis) of Pteraster tesselatus.

Regions numbered 1 and 2 show the two glandular areas of the supradorsal membrane: No. 1 shows the epidermis, covering the supradorsal membrane, with scattered gland cells; No. 2 indicates the bottom half of the supradorsal membrane which contains large gland sacs; No. 3 shows the location of the true epidermis.

Main canals (C) penetrate the supradorsal membrane and run from the surface to the nidamental chamber (NC). Smaller secretory canals run from a blind end into the main canals. These secretory canals are surrounded by gland cells which liberate their contents into the canals. The main canal and its secretory canals bound by non-glandular tissue comprise the one gland sac. The main type of gland cell which occurs in the gland sac is called a Gland Unit and has uniform secretory packets. The second type of gland cell surrounding the secretory canals are the B Type gland cells whose secretory packets have a dense sphere in their centre. The floor of the nidamental chamber, the true epidermis (3), is composed of two types of gland cells.

C, main canal; db, dermal branchiae; EE, external environment; NC nidamental chamber

The lines indicated by the letters A to E indicate sites of sections taken for the photomicrographs shown in Figs. 33 to 39 as follows:

A,Fig.39; B,Figs.34,36; C,Fig.38: D,Fig.37; E,Fig.33

(32)

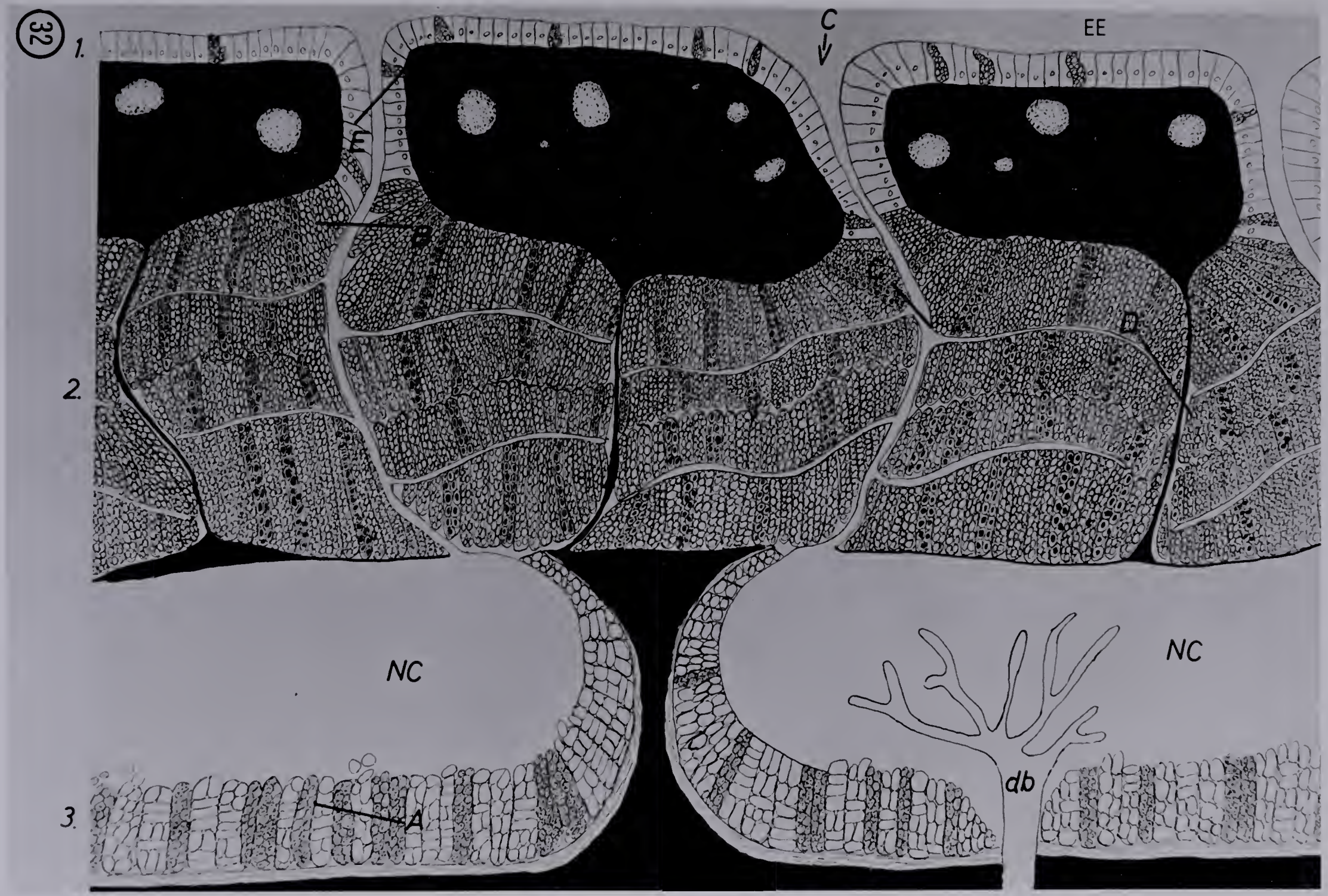


Fig. 33. This photomicrograph shows the epidermal covering of the supradorsal membrane on Pteraster tesselatus (Refer Fig. 32)

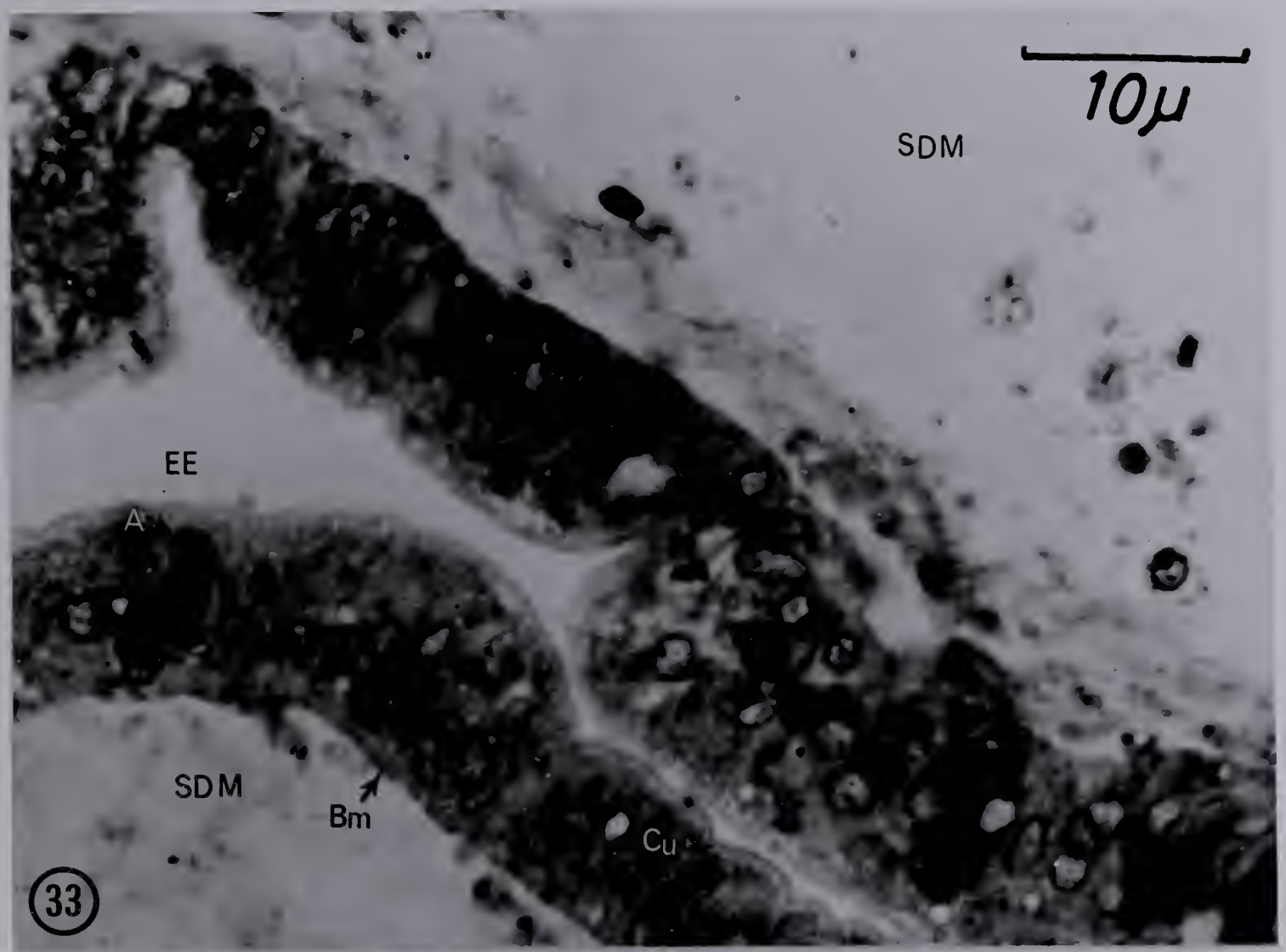
A, Type A gland cell; Bm, basement membrane; Cu, cuticle; EE, external environment; SDM, supradorsal membrane.

Bicarbonate/osmium fixation, Epon 812, embedding Richardson's staining.

Fig. 34. A canal (EE) from the surface of the supradorsal membrane is shown entering the canal system of the main gland complex which composes the bottom half of the supradorsal membrane (Refer Fig. 32).

EE(C), canal from the external environment; L, possible lipid deposit; Mg, main gland complex; SDM, supradorsal membrane.

SUSA fixation, Paraplast embedding, Alcian Blue staining.


10μ
SDM
EE
A
SDM
Bm
Cu
33

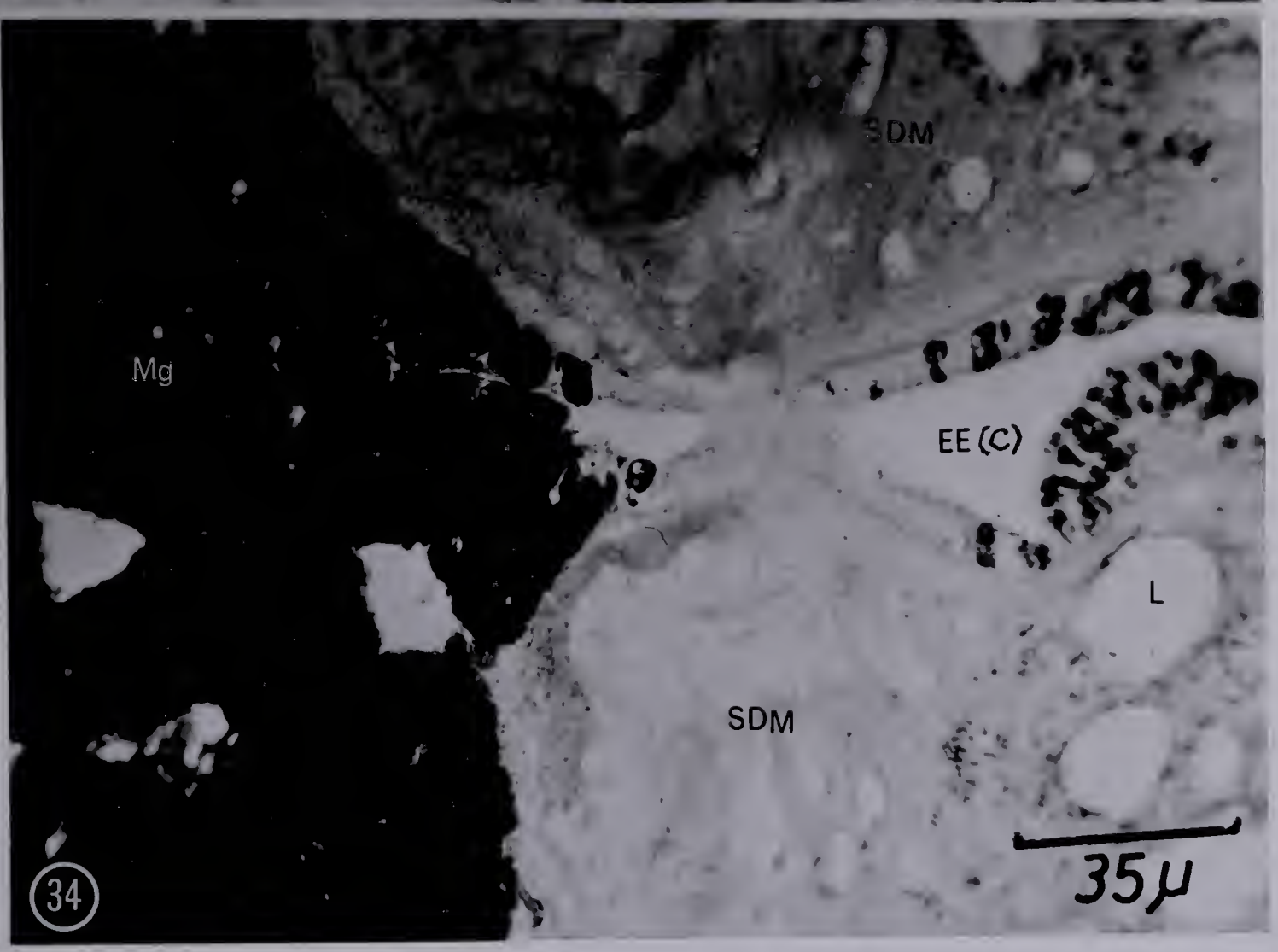

SDM
Mg
EE (C)
L
SDM
35μ
34

Fig. 35. Acridine Orange exhibiting fluoresence in the Type A gland cells. These gland cells are located in the epidermis of the supradorsal membrane.

SUSA fixation, Paraplast embedding, Acridine Orange stain.

Fig. 36. Acridine Orange exhibiting fluoresence in the main gland complex. (Refer Fig. 32).

EE(C), canal from the external environment; MG, main gland complex.

SUSA fixation, Paraplast embedding, Acridine Orange stain.

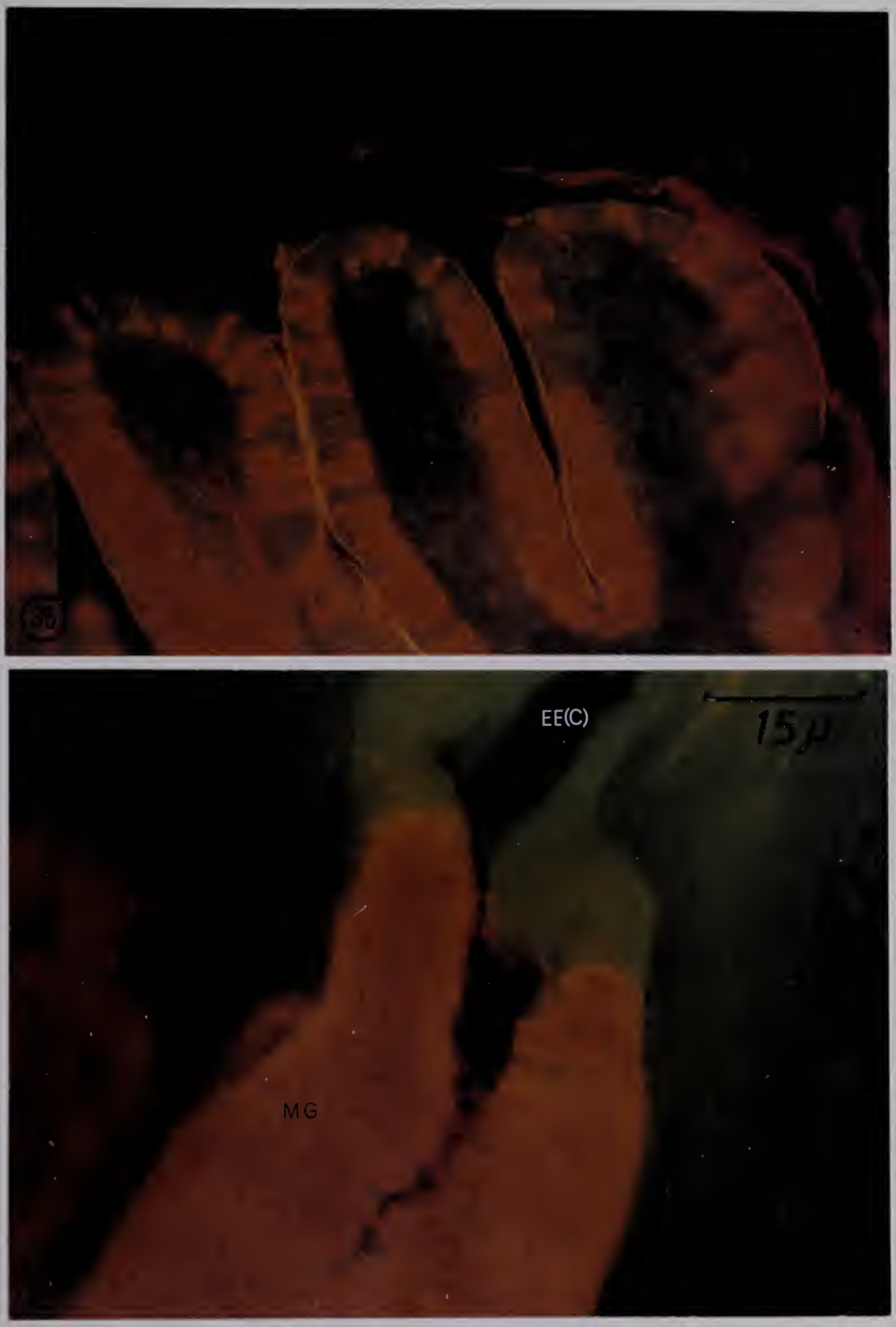

EE(C)
15μ
MG

Fig. 37 & Fig. 38 (Refer Fig. 32)

These two photomicrographs show the components of the main gland system in the supradorsal membrane of P. tesselatus. In Fig. 37, BD denotes the boundary of one component within the total complex. Each such basic component has one canal from the surface leading to it. Into this main canal run secondary canals, SC. Gland cells, or gland units (GU) release secretory packets (sp) which can move to the main canal. In Fig. 37, no packets have been released from the gland units into the secondary canal where as in Fig. 38 the secretory packets have been freed from the basic gland units. In Fig. 38 is also shown a gland cell of a different structural type, the Type B cell. This type of gland cell is similar to those previously discussed with the exception that it has secretory packets which appear clear except for a dense sphere in their centres.

sp, secretory packets; SC, secretory canal; BD, gland sac boundary; GU, gland unit; B, Type B gland cell.

Bicarbonate/osmium fixation, Paraplast embedding, Richardson's staining.

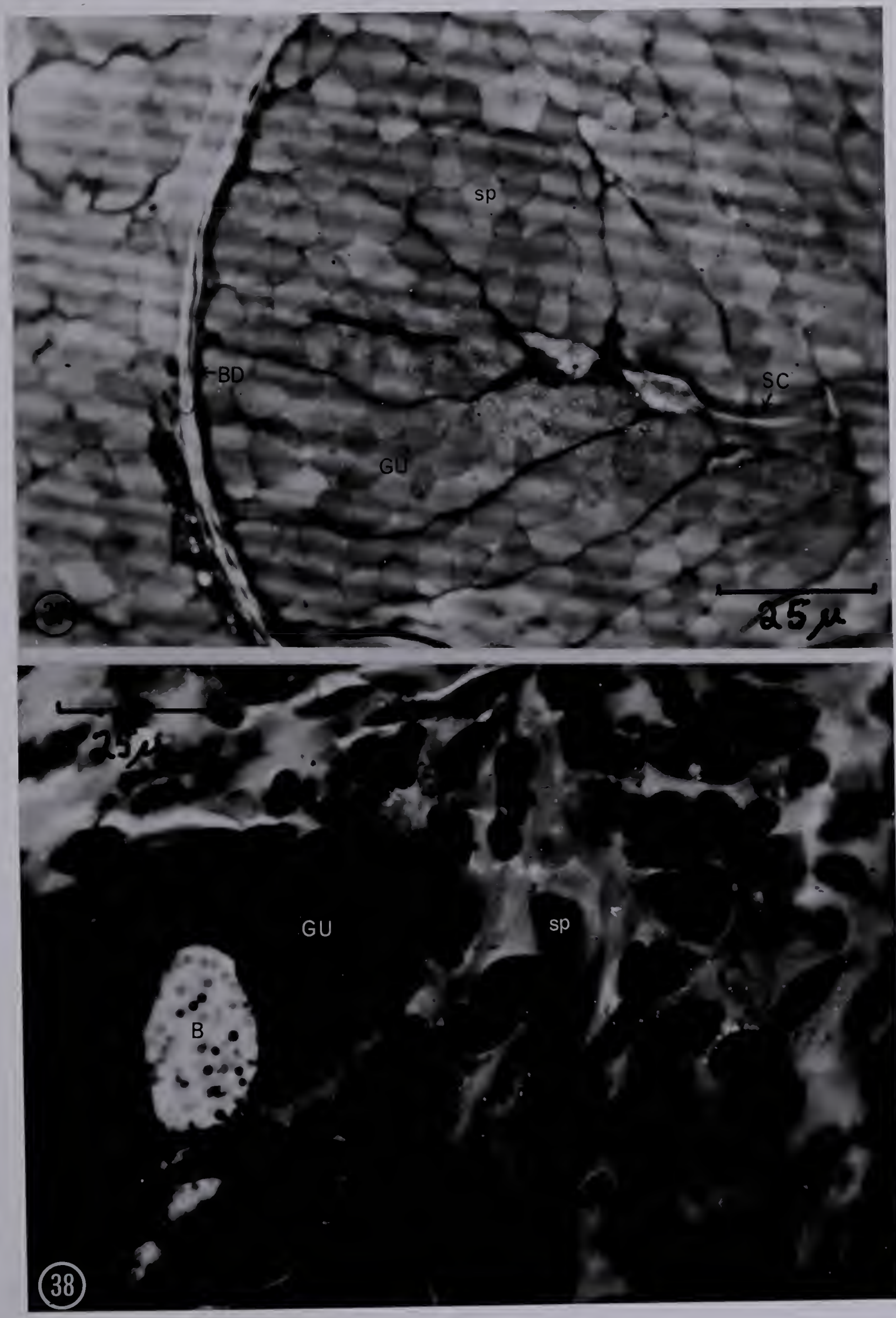

sp
BD
SC
GU
25μ
2.5μ
GU
sp
B
38

Fig. 39. This photomicrograph shows the cellular components of the true epidermis of Pteraster tesselatus. The true epidermis occurs on the floor of the nidamental chamber (Refer Fig. 32).

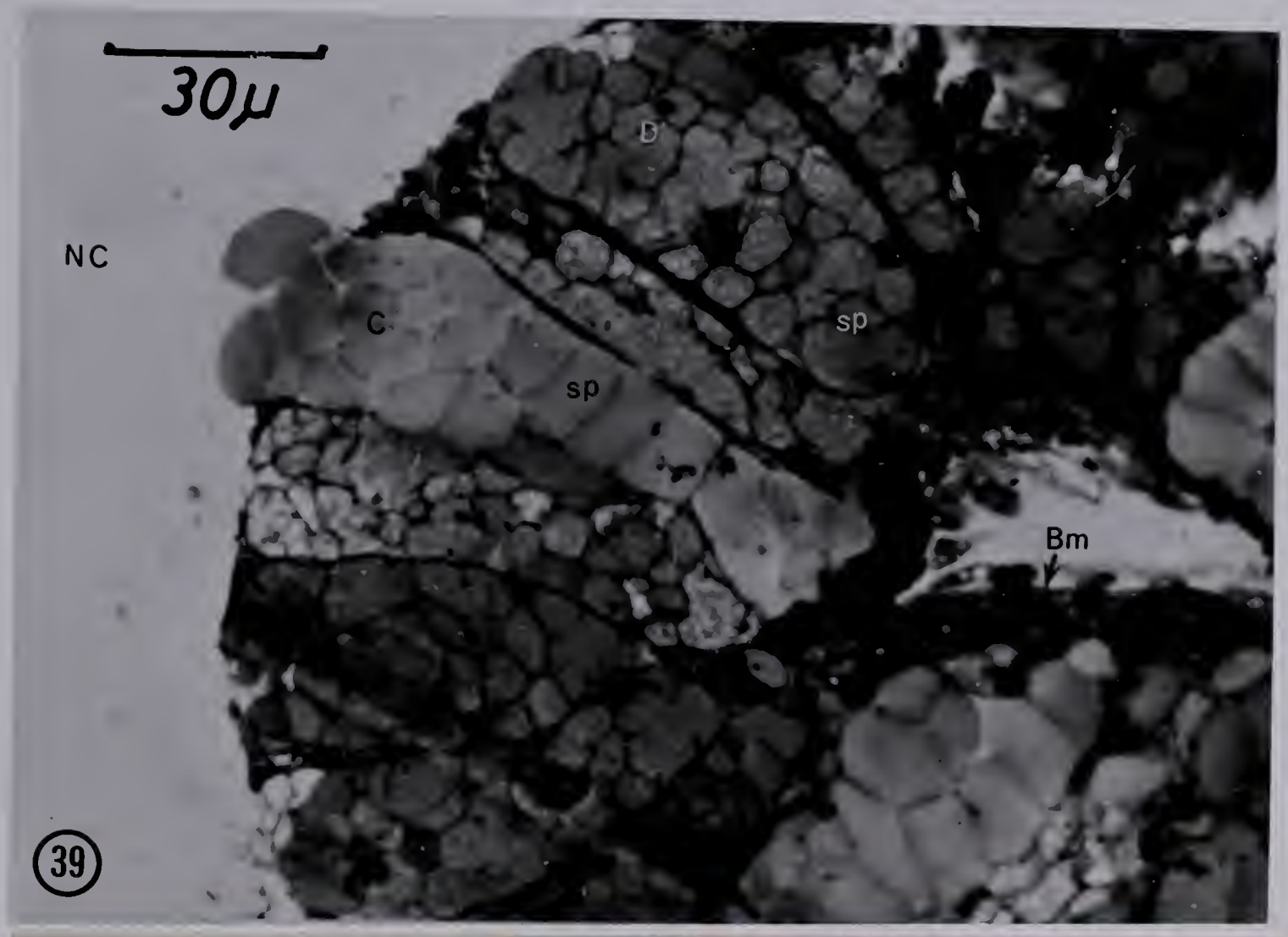
Bm, basement membrane; C, Type C gland cell; D, Type D gland cell; NC, nidamental chamber; sp, secretory packets.

Bicarbonate/osmium fixation, Epon 812 embedding, Richardson's staining.

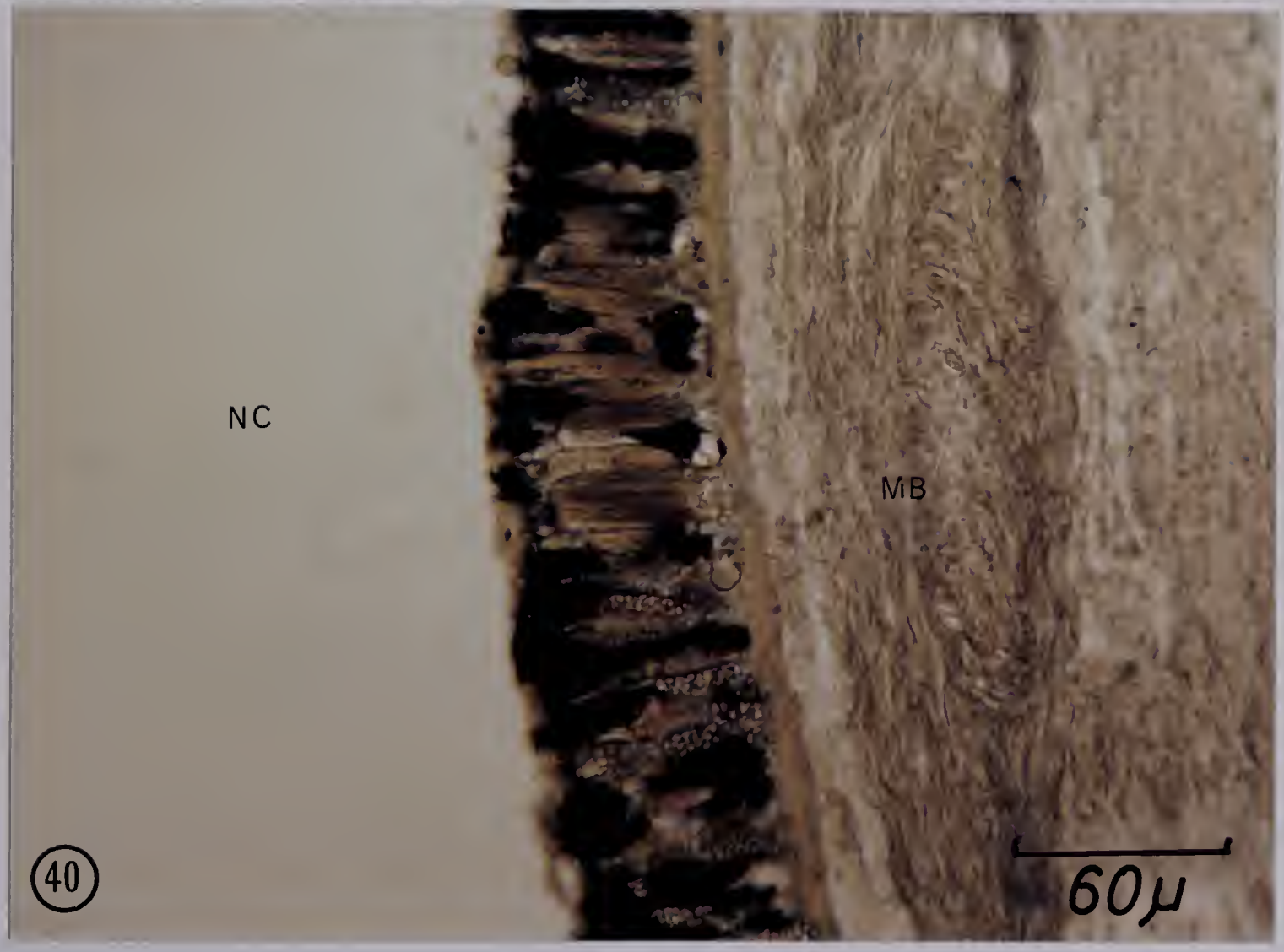
Fig. 40. True epidermis of Pteraster tesselatus showing the results of a histochemical test for acid mucopolysaccharide. Positive results show blue. "Mucus and Muriform" glands shown unstained. (Refer Fig. 32).

SUSA fixation, Paraplast embedding, Methylene Blue staining, pH 3.

MB, muscle bundle; NC, nidamental chamber.



39



40

Fig. 41. True epidermis of Pteraster tesselatus showing the results of a histochemical test for protein. Positive results show red. (Refer Fig. 32).

C, Type C gland cells; d, Type D gland cells; Mb, muscle bundle; NC, nidamental chamber.

SUSA fixation, Paraplast embedding, D.D.D. reaction with Fast Blue B salt.


NC
C
C
ML
50μ
41

F. Henricia leviuscula (Fig. 42)

H. leviuscula (Fig. 42) has a dense calcium endoskeleton over which lies a thin epidermal layer except where spicules emerge and are themselves exposed to the environment (Fig. 43, Fig. 47). The ridges or groups of surface spines displayed dorsally have a series of smaller protuberances which terminate in spicules (Fig. 43). The epidermis covering the area between the spicules is of varying depth, is ciliated and is covered dorsally by a uniform cuticle. Nuclei are prominent, but distinct cellular boundaries are not observed except in gland cells.

The most common type of gland cell which occurred in H. leviuscula was Type A (Fig. 44 & 45) (Table 6). This type was most common forming the deep epidermis around the dermal branchiae. It was composed of distinct secretory packets with very little cytoplasm and a prominent nucleus in its lower portion. The secretory packets were released to the exterior through a secretory pore (Fig. 44). This Type A gland cell was found to contain acid mucopolysaccharide. A similar type of gland cell was observed in the epidermis around the small paxillae. These cells differed only in that they had prominent necks leading to the surface.

A second type of gland cell was observed in conjunction with the Type A cell. Type B gland cells (Fig. 45) (Table 6) were characterized by prominent nuclei, small secretory

vesicles, and the absence of openings to the surface. Histochemical tests showed no apparent reactions for this type of gland cell.

In the areas where these two types of gland cell occurred, the P.A.S. reaction showed few positive reactions on unknown gland cells.

The third type of glandular structure in H. leviusscula was first observed by Mortensen and Lieberkind (1928), and more recently by Hayashi (1935). In both cases it was described as a single gland cell. When this structure was looked at with Epon 812 embedded sections, it was found to be composed of two distinct cellular units in a glandular matrix. The complete unit, or glandular sac, (Fig. 46) (Table 6) is a pouch which extends far below the epidermal region and is surrounded by the connective tissue of the body. The contents of the glandular sac was easily distinguishable from the epidermal glandular tissue (Fig. 47). The components of the glandular sac appeared to be distinct nucleated structures, with secretory packets filling them and without any cytoplasm in evidence. The first component, G_1, (Fig. 48) resembles the Type B gland cell in every way except its location. The second component, G_2, resembles G_1 except that it has less distinct secretory packets and an affinity for Richardson's stain whereas G_1 does not (Fig. 49). The structure of the G_3 gland tissue has not been resolved. It may contain a series of individual cells,

the walls of which do not pick up Richardson's stain, or it could be a syncytium.

Histochemical tests have not been run on this tissue.

TABLE VI

COMPARATIVE DATA ON THE ABORAL GLAND CELLS OF HENRICIA LEVIUSCULA

		Gland Type A	Gland Type B	Gland Pocket G_1	G_2	G_3
length		83 μ	83 μ	7 μ	7 μ	?
width	base	10 μ	10 μ	7 μ	7 μ	?
	neck	2 μ	0.8 μ	--	--	?
secretory particle diameter		2.8 μ	.5 μ	.5 μ	.5 μ	1 μ
chemical composition of gland cell		acid mucopoly-saccharide and P.A.S. positive material	no reaction	?	?	?
opening to exterior		yes, pore	yes, pore	?	?	yes, pore
approximate ratio in the epidermis		50%	50%	?	?	?
location of nucleus		base	base	?	?	?
distribution in epidermis		in epidermis around spines	in epidermis around spines	?	?	?
Remarks						

Fig. 42. The aboral surface of Henricia leviuscula. .
Dried specimens. X ½

Fig. 43. The aboral surface of Henricia leviuscula. .
x4.

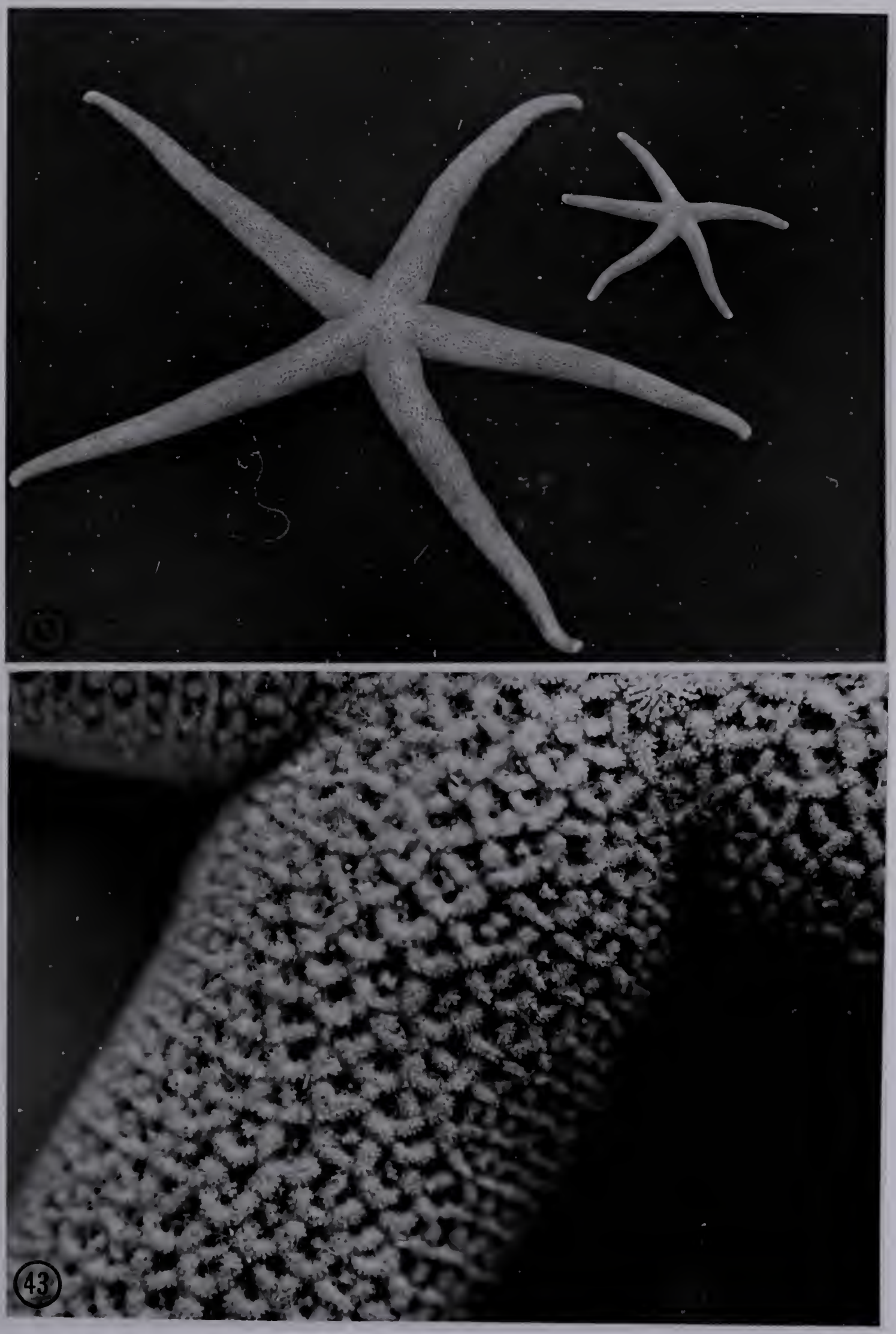


Fig. 44. Type A gland cell showing its secretory pore in the glandular interpaxillary region of Henricia leviuscula.

A, Type A gland cell; Ca, site of calcium spicule; EE, external environment; Sp, secretory pore; N, nucleus.

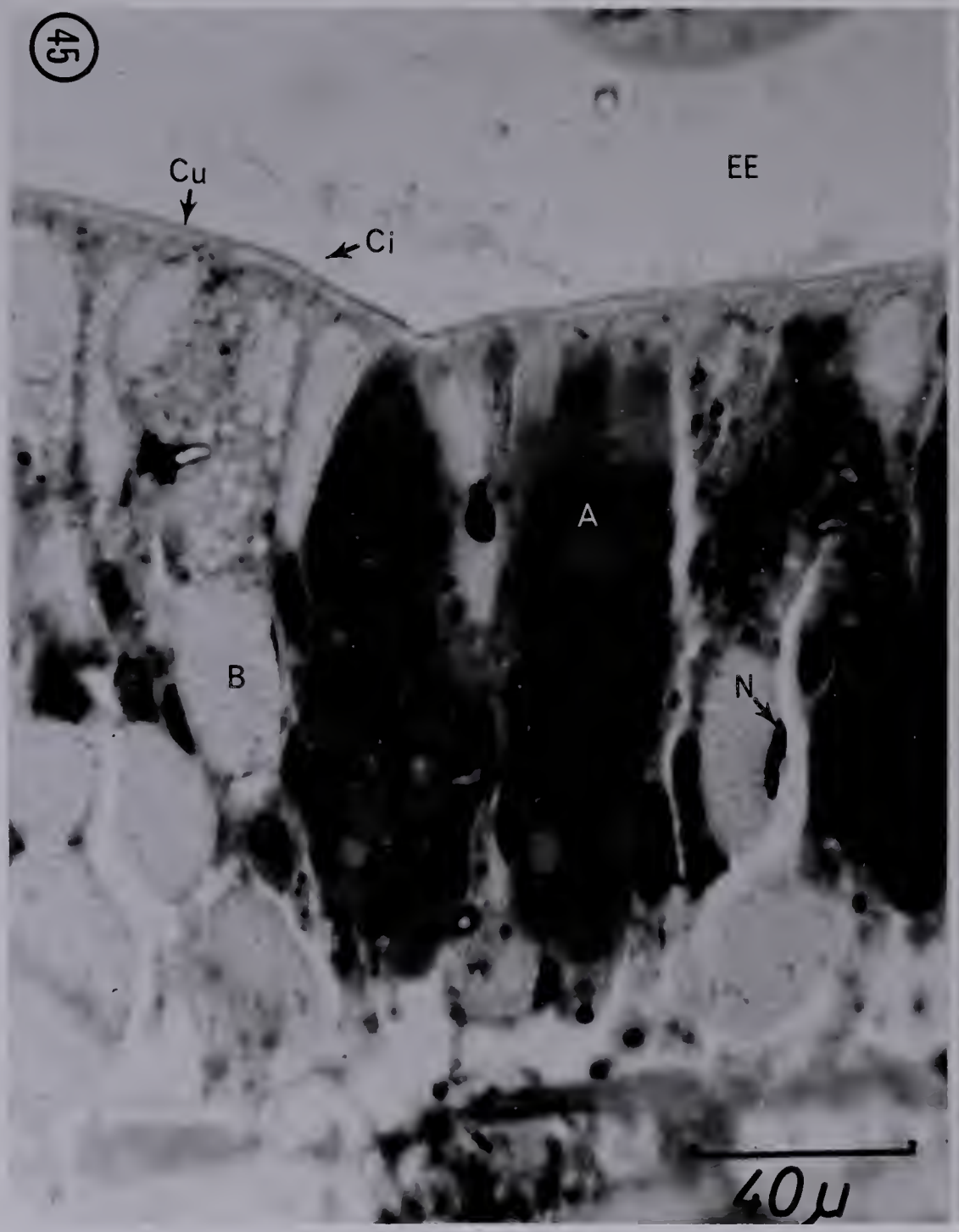
Bicarbonate/osmium fixation, Epon 812 embedding, Richardson's staining.

Fig. 45. Typical glandular complement found in the interpaxillary regions of Henricia leviuscula.

A, Type A gland cells; B, Type B gland cells; Cu, cuticle; Ci, cilia; EE, external environment.

Bicarbonate/osmium fixation, Epon 812 embedding, Richardson's staining.

45



44

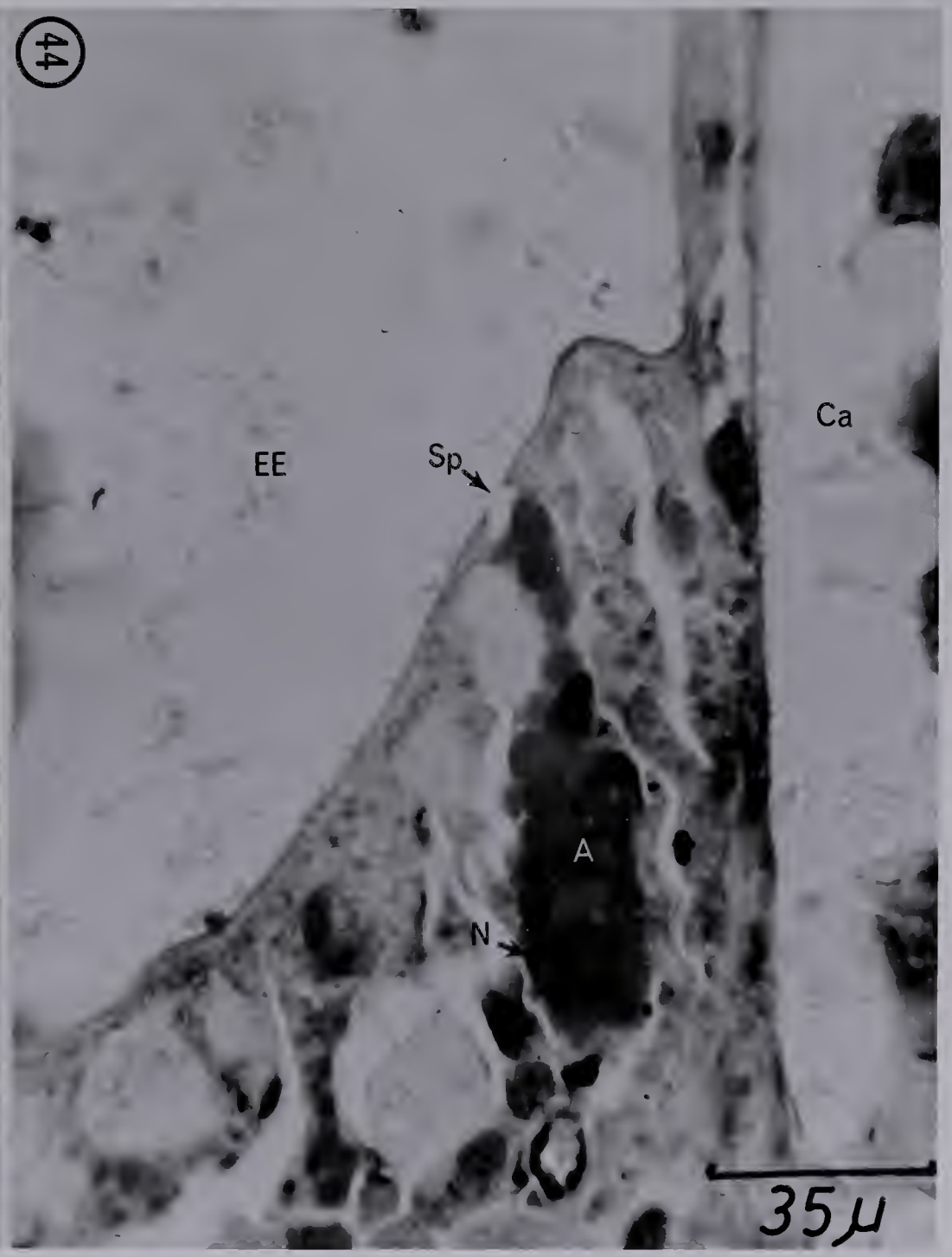


Fig. 46. Section through the aboral surface of Henricia leviuscula showing the large glandular pocket which is unique to the species stained.

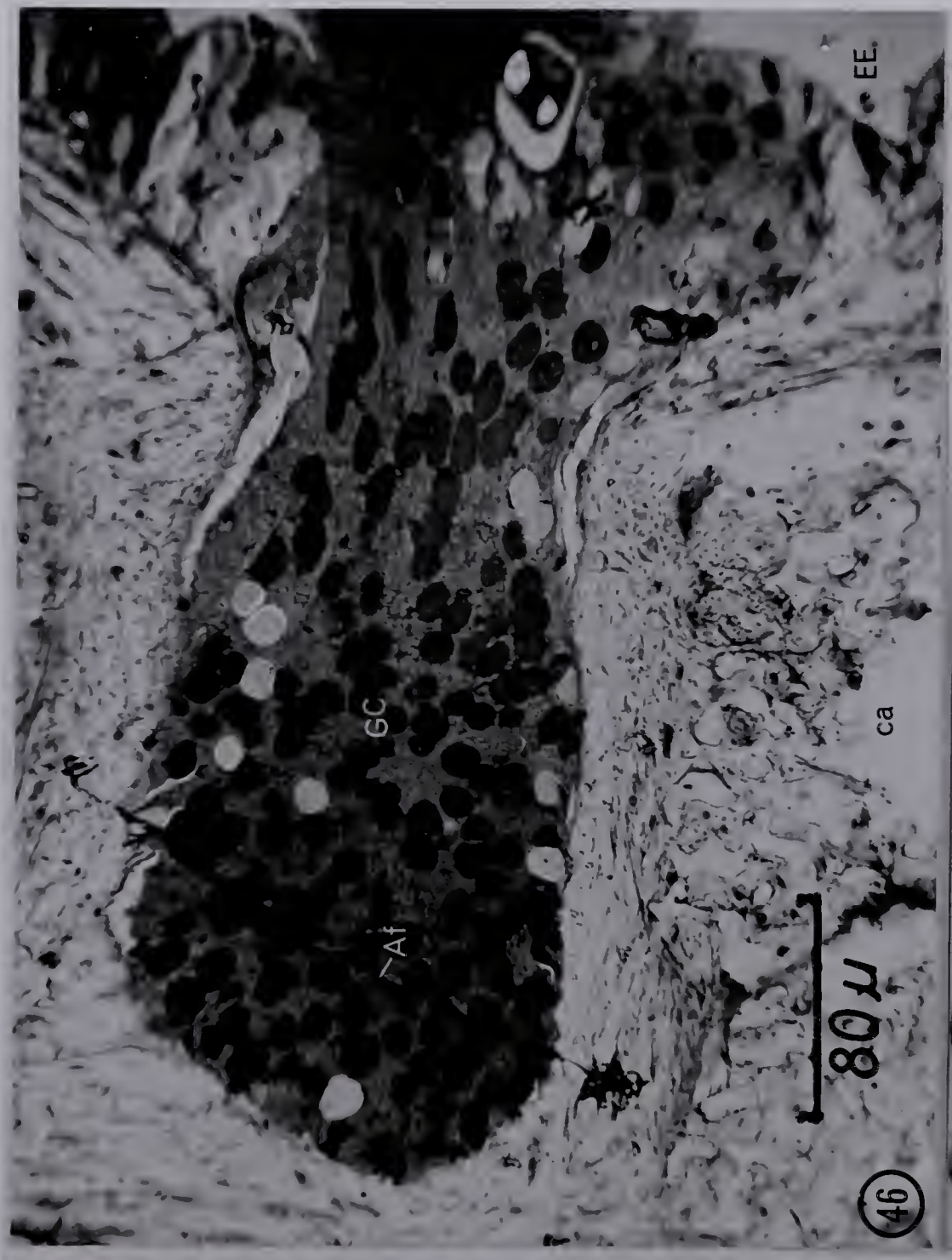
Af, artifact; Ca, site of calcium deposit, EE, external environment; GC, glandular pocket.

Bicarbonate/osmium fixation, Epon 812 embedding, Richardson's staining.

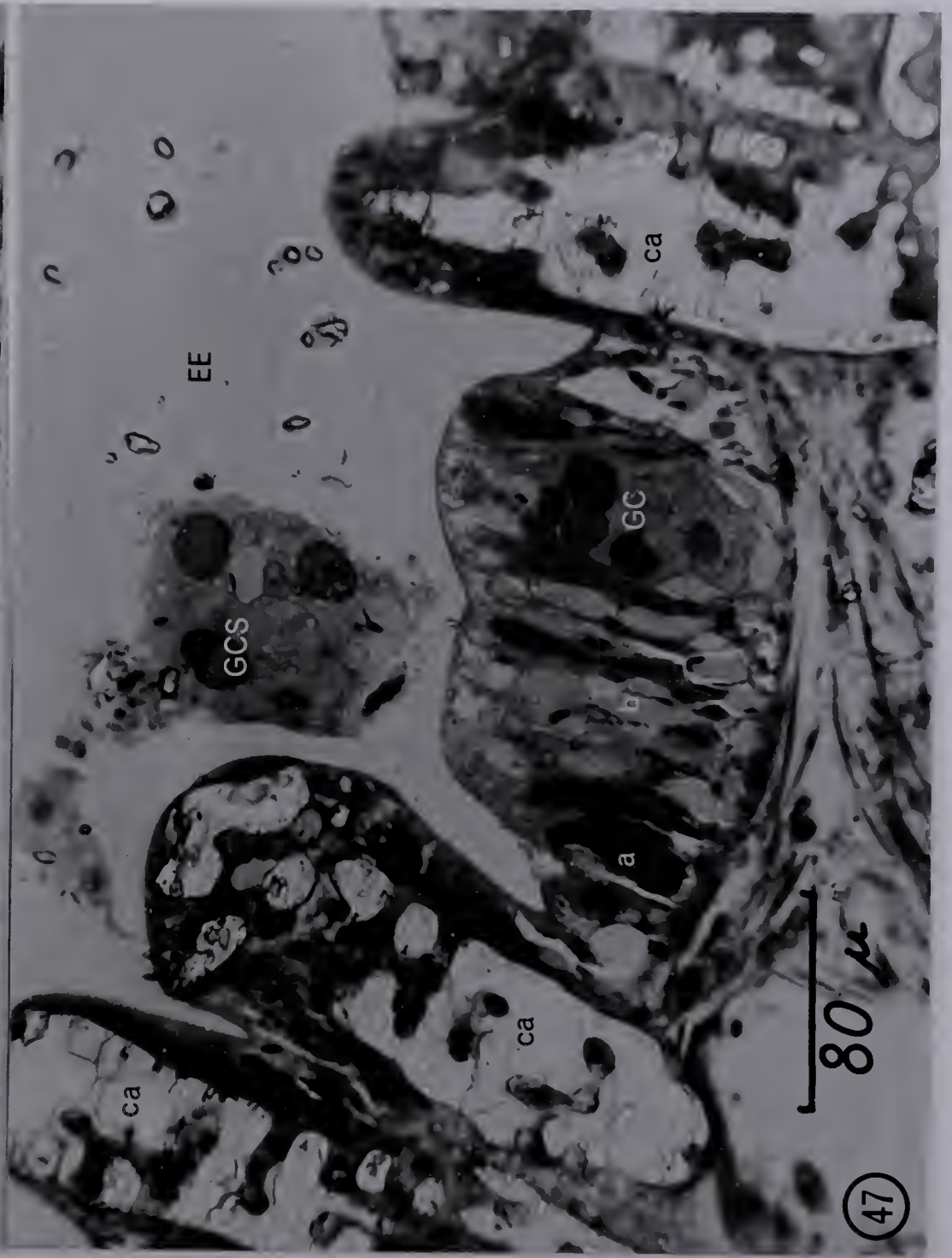
Fig. 47. Typical cross section of Henricia leviuscula epidermis showing the glandular pocket between the calcium spines. Also shown is an edge of the glandular pocket, and some of its secreted contents.

a, Type A gland cell; b, Type B gland cell; ca, site of calcium spicule; EE, external environment; GC, glandular pocket; GCS, glandular pocket secretion.

Bicarbonate/osmium fixation, Epon 812 embedding. Richardson's staining.



(46)



(47)

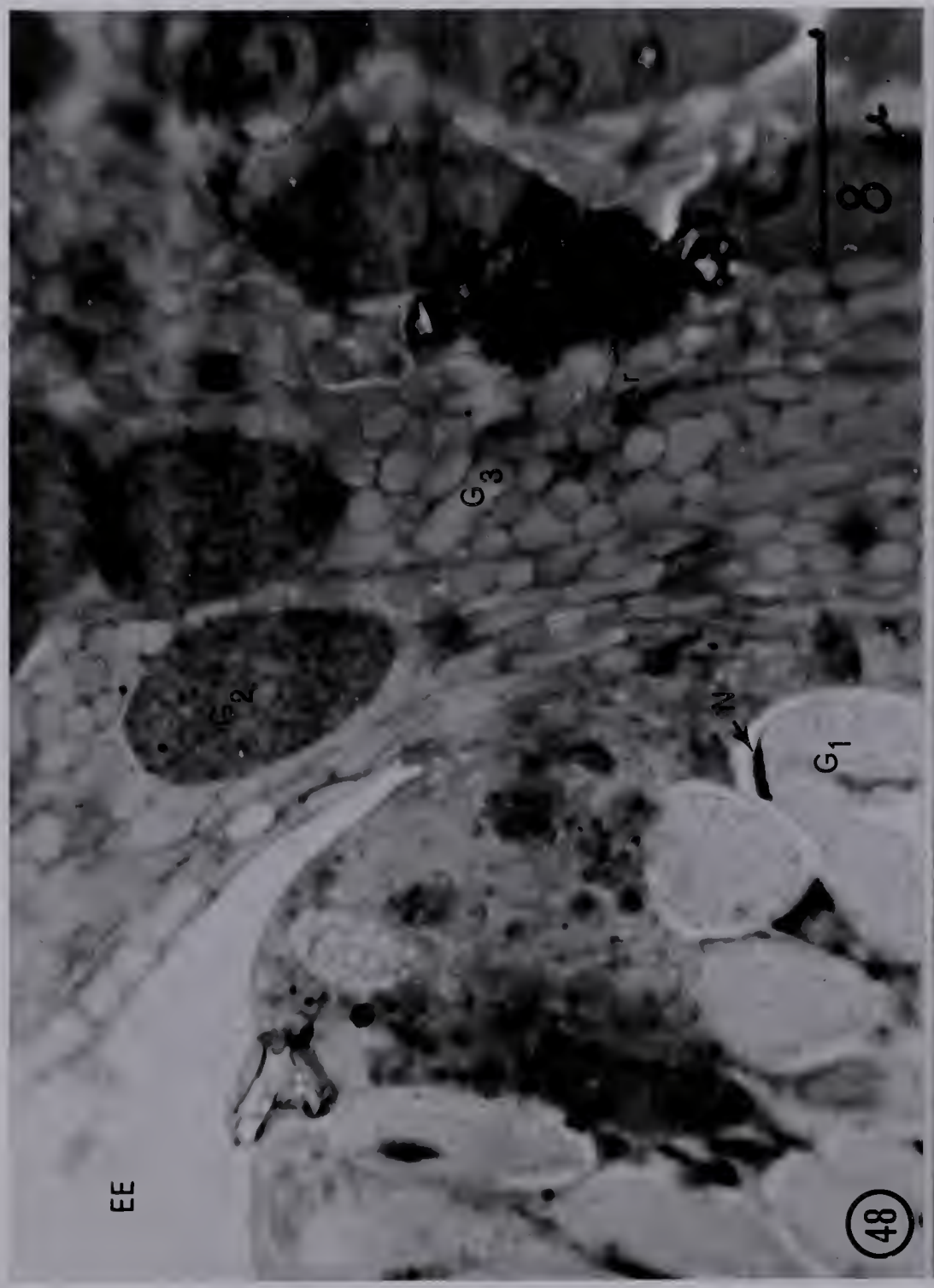
Fig. 48. The edge of the glandular pocket is shown at the point where its secretion products pass through the epidermis and are liberated to the environment. Note the three distinct gland types present in the glandular pocket, G_1 , G_2 , G_3 .

Ar, Artifact; EE, external environment; G_1, gland cell type; G_2, gland cell type; G_3, gland cell type; N, apparent nucleus.

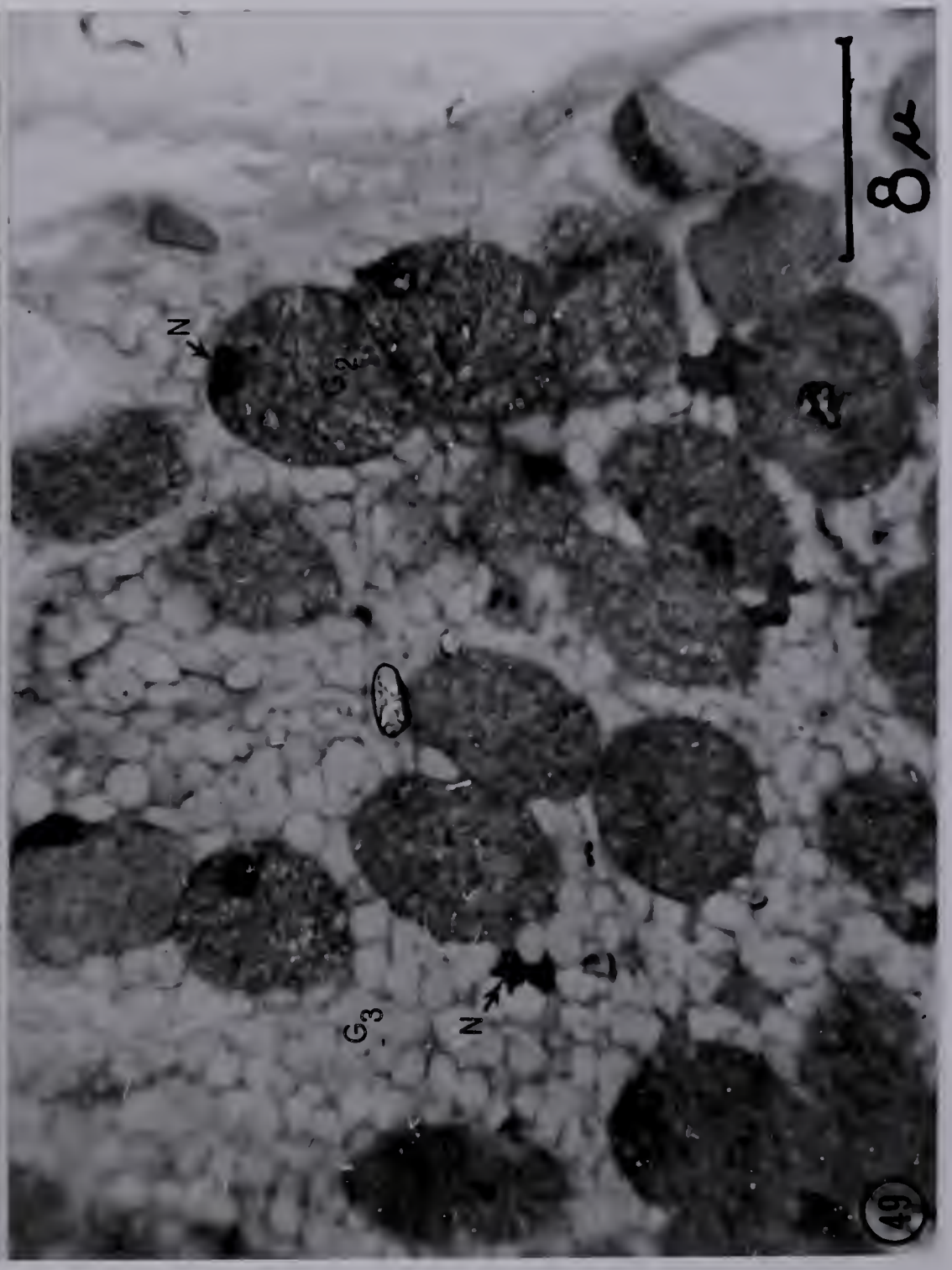
Bicarbonate/osmium fixation, Epon 812 embedding, Richardson's staining.

Fig. 49. T.S. shows the bodies appearing to be nuclei present in the gland cell types G_2 and G_3.

G_2, gland cell type; G_3, gland cell type; N, presumed nuclei.



48



49

G. Pycnopodia helianthoides

P. helianthoides (Fig. 50) has an aboral epidermis which is characterized by large pompom topped paxillae, around which are a large number of branched dermal branchiae and also pedicellariae. (Fig. 51) (Page 22). P. helianthoides has no cilia present on its aboral surface. The epidermis of this animal is approximately 40 μ in depth and rests on a basement membrane which sharply separates it from the sub-epidermal tissue (Fig. 52, 53 & 54). The epidermis is composed almost entirely of gland cells, except where the dermal branchiae occur. In this area the gland cells are found randomly scattered among columnar epithelium. The surface of the epidermis is covered by a distinct cuticle (Fig. 52), which appears to be composed of an outer dense layer and an inner less dense layer.

Four types of gland cell have been observed in P. helianthoides which will be called A, B, C, and D for convenient identification.

The Type A gland cell is by far the most common (Fig. 54) (Table 7). The small secretory packets produced by this gland are selectively contained in the upper half of the cell body, below which occurs a dense cone shaped structure with the base adjacent to the secretory packets (Fig. 52), the nucleus, and the cytoplasm of the cell. This gland is unusual in that it does not have an exit in spite of its close prox-

imity to the surface. Cells of Type A did not respond to any of the histochemical tests performed. Therefore, their chemical composition could not be determined.

Gland cells of type B were the second most common gland cell type found in P. helianthoides (Fig. 52) (Table 7). They lie in close proximity to the gland cells of Type A but are easily distinguished from the latter by the fact that each have a distinct pore to the surface and that they contain large irregular secretory packets filling the entire cell body (Fib. 52, Fig. 54). Histochemical tests showed that this gland cell contained either acid mucopolysaccharide (Fig. 56) or a P.A.S. positive substance probably a neutral mucopolysaccharide (Fig. 55) and in some cases both compounds were found in the same cell.

Cells of Type C (Fig. 53) (Table 7) occurred less commonly than did those of Types A and B. They resembled Type B in that they opened to the surface by a pore in the cuticle layer. Their secretory contents also fill the entire cell as in Type B. They differ from Type B, and all other gland cells studied, in that they do not have secretory packets. Instead their secretory contents take the form of loosely coiled ribbon-like structures which exhibit a high degree of metachromasia in sections cut from Epon 812 embedded tissue and stained by Richardson's method. As this is not known to be a histochemically valid identification and this structure cannot be correlated with

Paraplast sections due to lack of structural definition, the chemical composition of these cells could not be determined.

The fourth type of glandular cell in P. helianthoides Type D, (Fig. 52) (Table 7) is rare. These cells exhibit an opening to the surface and they show distinct secretory packets running the full length of the cell body. These packets stain orthochromatically blue with Richardson's stain after Epon 812 embedding. The packets themselves are different from those found in the other gland cells in P. helianthoides in that they are far more spherical and appear to retain this characteristic even when tightly packed. The chemical composition of these cells could not be determined for the same reason that applied to gland type C.

TABLE VII

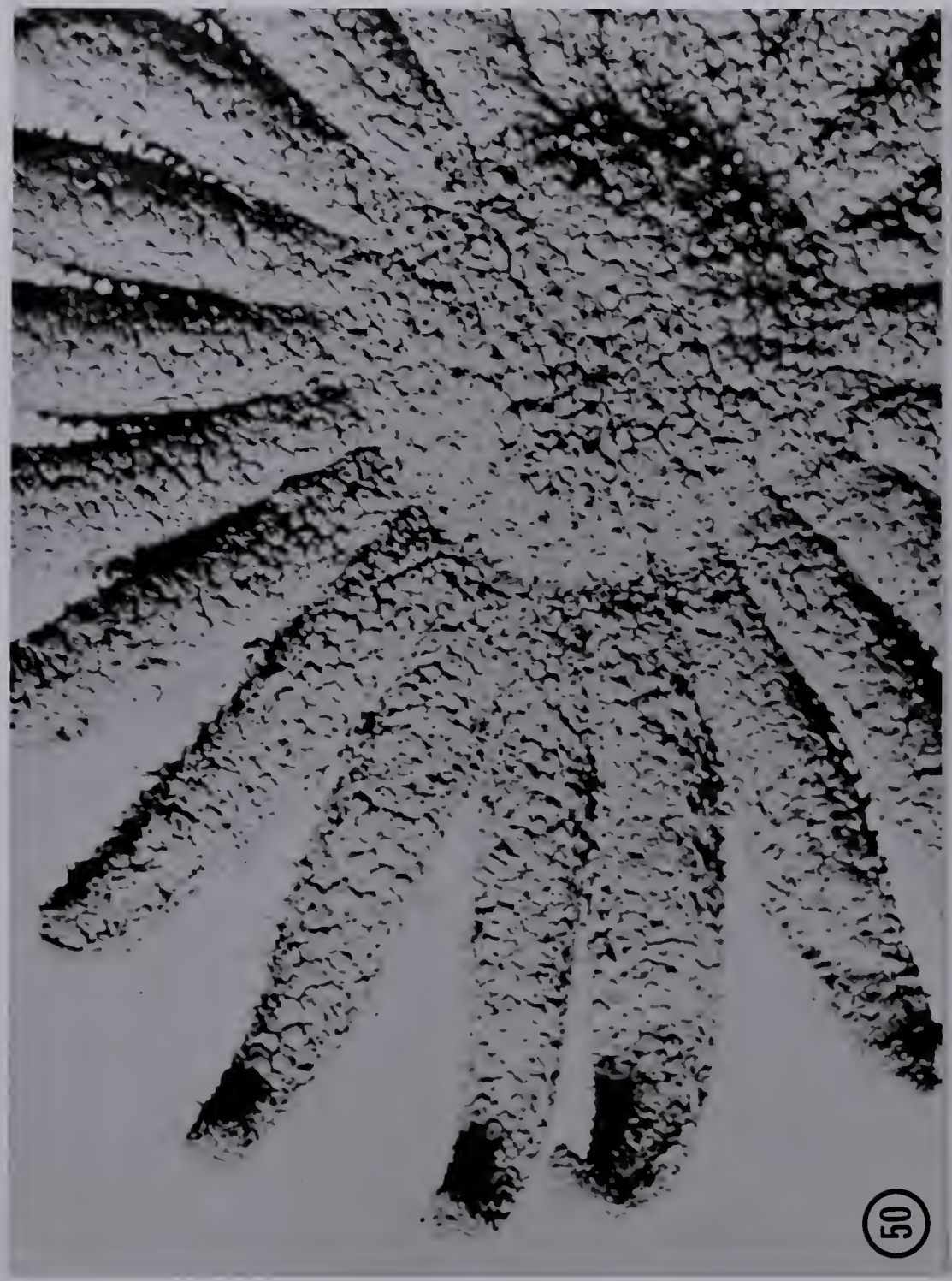
COMPARATIVE DATA ON THE ABORAL GLAND CELLS OF PYCNOPODIA HELIANTHOIDES

		Gland Type A	Gland Type B	Gland Type C	Gland Type D
length		40 μ	40 μ	40 μ	40 μ
width	base	7 μ	10 μ	10 μ	10 μ
	neck	7 μ	2 - 4 μ	2 - 4 μ	--
secretory particle diameter		2 μ	8 μ	n/a	2 - 8 μ
chemical composition of gland cell		no reaction	acid muco-polysaccharide and/or P.A.S. positive material	?	?
opening to exterior		no	yes, pore	yes, pore	yes, pore
approximate ratio in epidermis		60%	40%	1%	1%
location of nucleus		below secretory packets	base	base	?
distribution in epidermis		entire aboral surface	entire aboral surface	entire aboral surface	entire aboral surface
Remarks			range of histo-chemical reactions	may be continuous thread	

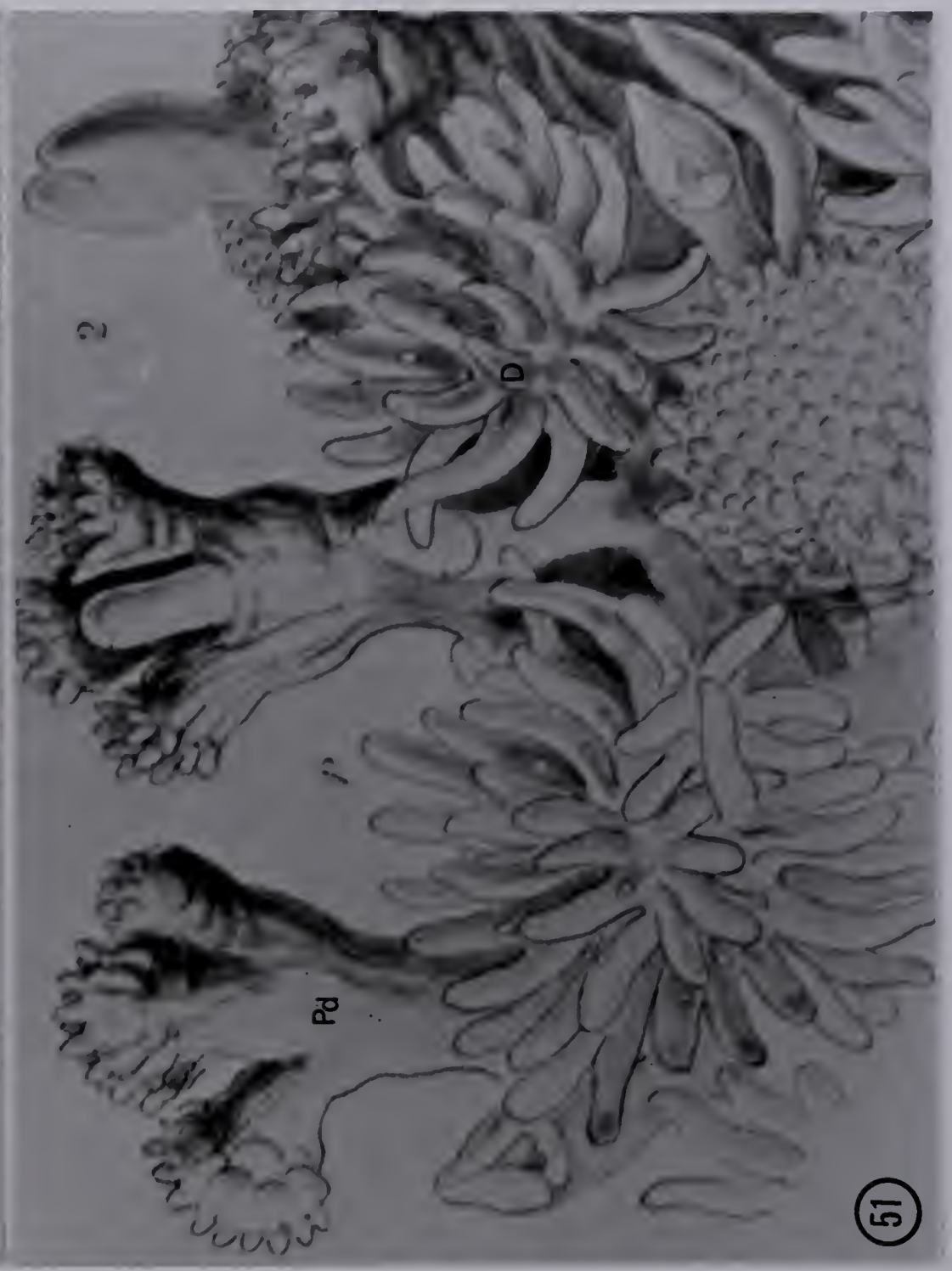
Fig. 50. The aboral surface of Pycnopodia helianthoides, taken from a picture in the work by Fisher, 1930. X ¼.

Fig. 51. A semi-diagramatic drawing illustrating the aboral surface of Pycnopodia helianthoides taken from the work by Fisher, 1930. X 20.

D, dermal branchia, Pd, paxilla.



50



51

Fig. 52. Cross section through the aboral epidermis of Pycnopodia helianthoides.

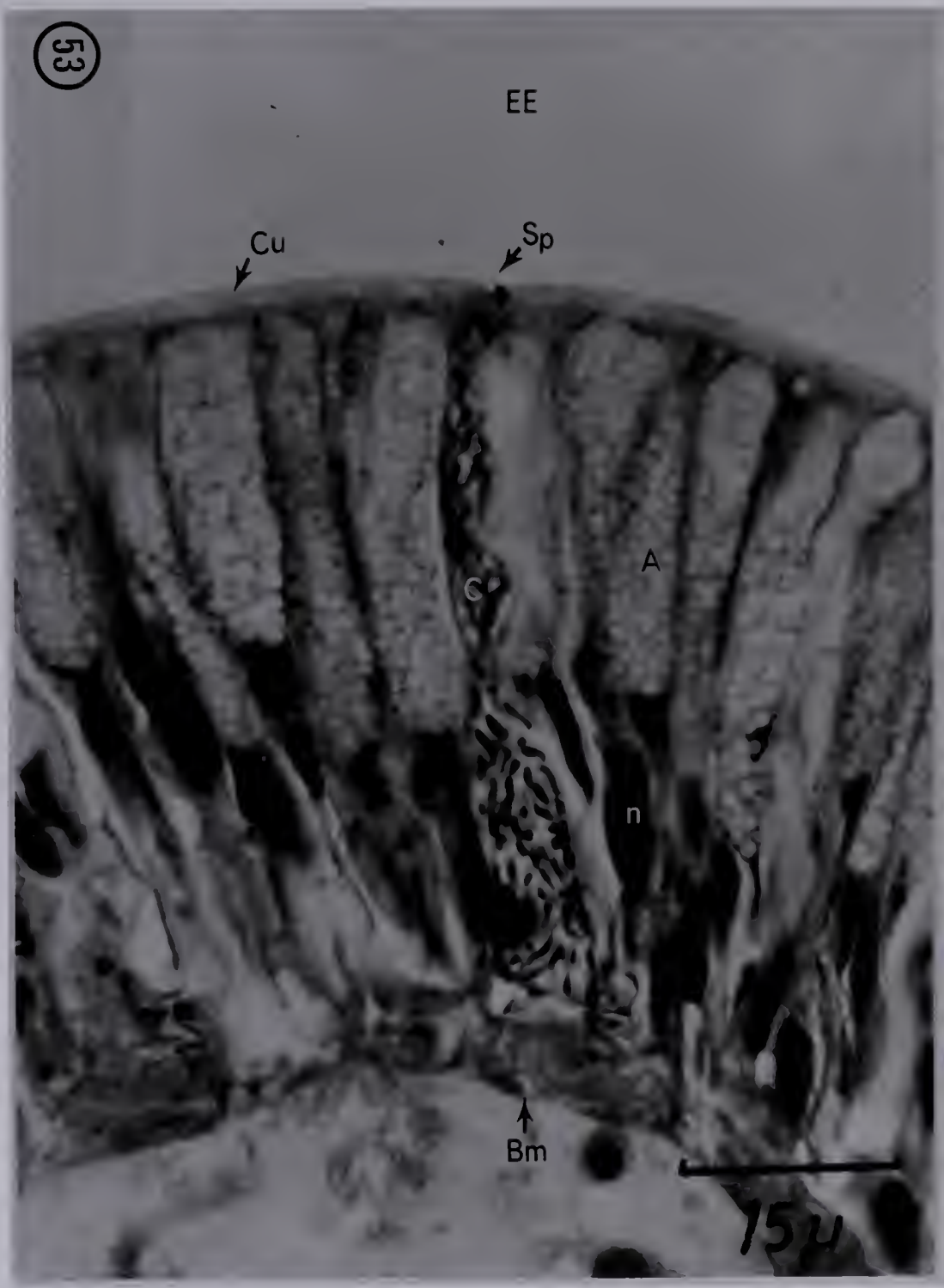
A, Type A gland cell; B, Type B gland cell; Bm, basement membrane; cu, cuticle; D, Type D gland cells; EE, external environment; n, nucleus; X, non-nuclear structure below secretory packets.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's stain.

Fig. 53. Cross section through the aboral epidermis of Pycnopodia helianthoides.

A, Type A gland cells; Sp, secretory pore; C, Type C gland cells; Cu, cuticle; EE,external environment; N, nucleus; Sp, secretory pore.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's stain.


53
EE
Cu
Sp
A
G
n
Bm
15μ

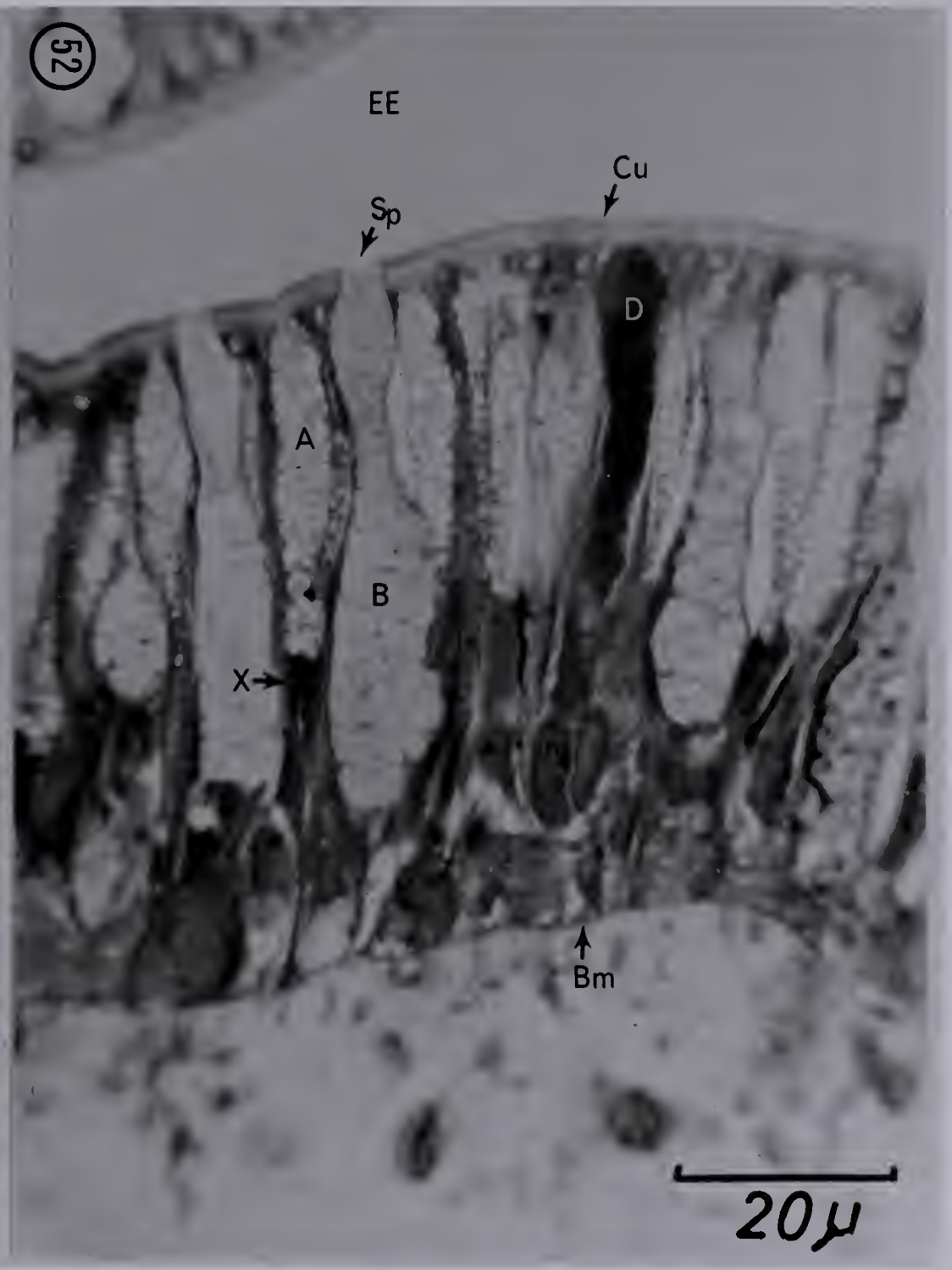

52
EE
Cu
Sp
D
A
B
X
Bm
20μ

Fig. 54. Cross section through the aboral epidermis of Pycnopodia helianthoides.

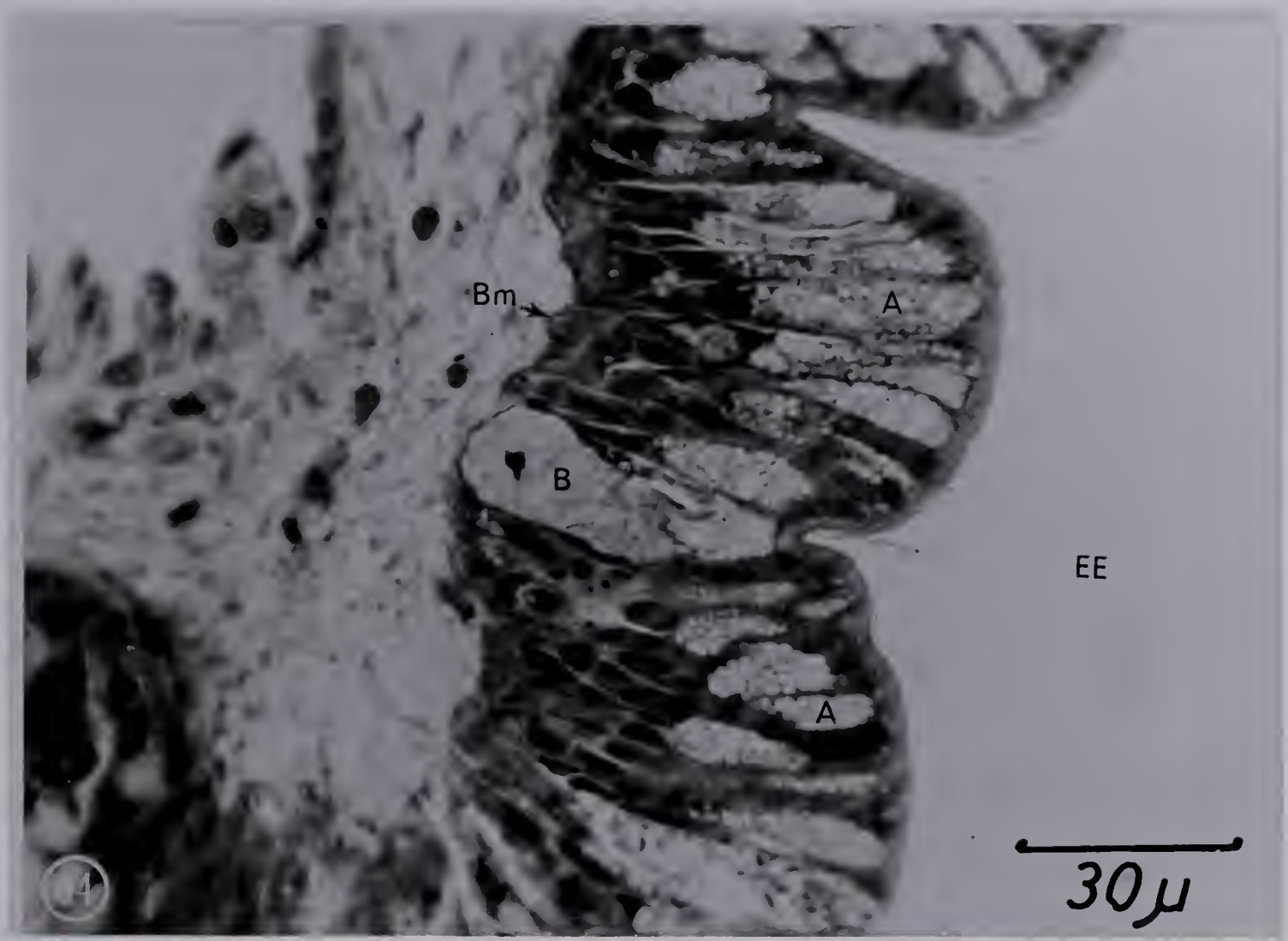
A, Type A gland cells; B, Type B gland cells; Bm, basement membrane, EE, external environment.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's staining.

Fig. 55. Cross section through the aboral epidermis of Pycnopodia helianthoides showing a positive P.A.S. response in the gland cells.

Arrows indicate P.A.S. positive gland cells.

SUSA fixation, Paraplast embedding, P.A.S. test.


Bm
A
B
EE
A
30μ
4

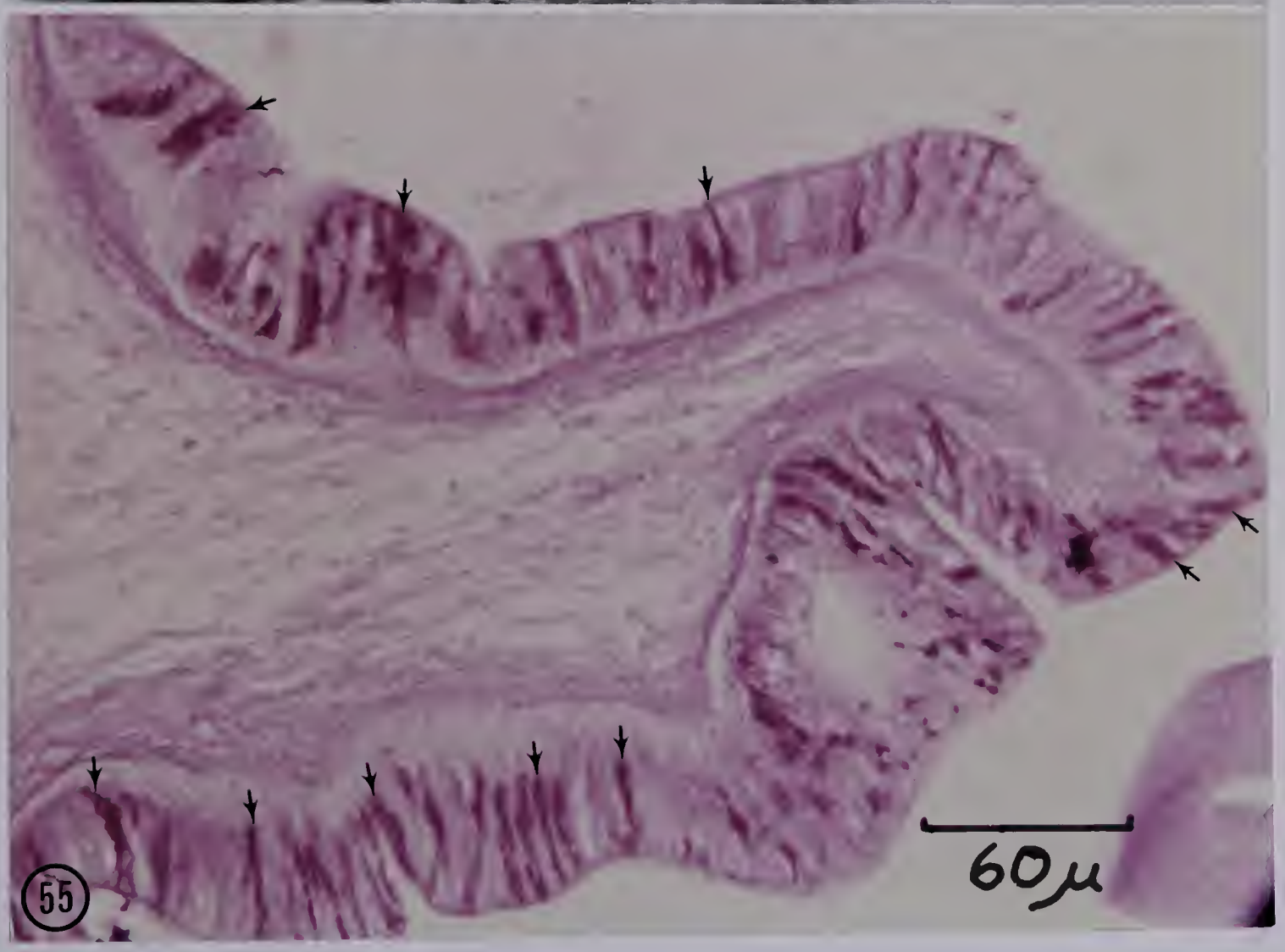

60μ
55

Fig. 56. A cross section through the aboral surface of the aboral epidermis of Pycnopodia helianthoides showing Acridine orange fluorescence in gland cells indicating acid mucopolysaccharide content.

SUSA fixation, Paraplast embedding, Acridine orange stain.

H. Leptasterias hexactis

L. hexactis has numerous calcareous spines protruding through the aboral surface, surrounded by dermal branchiae and pedicellariae. The epidermal layer covers most of the aboral surface except where it is pierced by a calcium spine and on the tips and interior of pedicellariae. This epidermis took the form of a distinct ciliated columnar epithelium whose upper most part appeared to be covered by a cuticle which was composed of three parts; a thin, dense outer layer, a less dense thin second layer, and a thick deeper layer of medium density. A distinct division occurs between the epidermal tissue and the underlying tissue, but no distinct basement membrane could be seen with the light microscope.

Three gland types have been observed in L. hexactis. The most common gland cells, to be known as Type A (Fig. 58) (Table 8) were found to secrete acid mucopolysaccharide. Type A appears to be at the edge of the cell base with a small amount of cytoplasm around and behind it. The most characteristic structural point in the cell is the irregularity of the large secretory vesicles which fill almost the entire cell body. These packets appeared in a crinkled state with apparent bits of cytoplasm between them. This condition appeared in cells which were apparently mature and in some cases seemed to have been actively secreting when fixed.

Gland cells of the second type, Type B, (Fig.58) (Table 8) are found only about half as frequently as those of Type A. This type of gland cell extends the full length of the epidermis in which it is present, but the secretory vesicles it contains occur in the top half of the cell exclusively. The secretory vesicles show no staining reaction when stained with Richardson's after sections had been cut from Epon 812 embedded material. No external pore appears to be present and no histochemical reaction has been observed for these cells.

The gland type designated as C occurs in the epidermis with about the same frequency as does Type B. In this gland cell, as in Type B, the secretory packets occur in the top half of the cell, while the nucleus and cytoplasm occur in the lower half of the cell. In this case the secretory packets stain lightly orthochromatically with Richardson's stain. Due to the fact that the secretory packets occupy the top half of the cell, and have an affinity for Richardson's stain I believe that this gland cell may be responsible for the positive DDD reaction shown by this tissue. This tissue when stained with One Step Mallory showed that the Orange G component selectively stained the same structures that showed positive with the DDD reaction (Fig. 59). However, it is not possible when viewed as a section cut from paraffin embedded tissue to state at this time that the Type C gland cell is responsible for the positive DDD reaction when the Type B gland cell has the same

structure.

A fourth type of gland cell (Type D) was occasionally seen in L. hexactis but it was not possible to do a detailed study on it. It was detected in Epon 812 embedded sections by secretory packets that ran the full depth of the cell and by the intense orthochromatic staining of the packets. The secretory packets also took the form of perfect spheres which were not deformed.

TABLE VIII

COMPARATIVE DATA ON THE ABORAL GLAND CELLS OF LEPTASTERIAS HEXACTIS

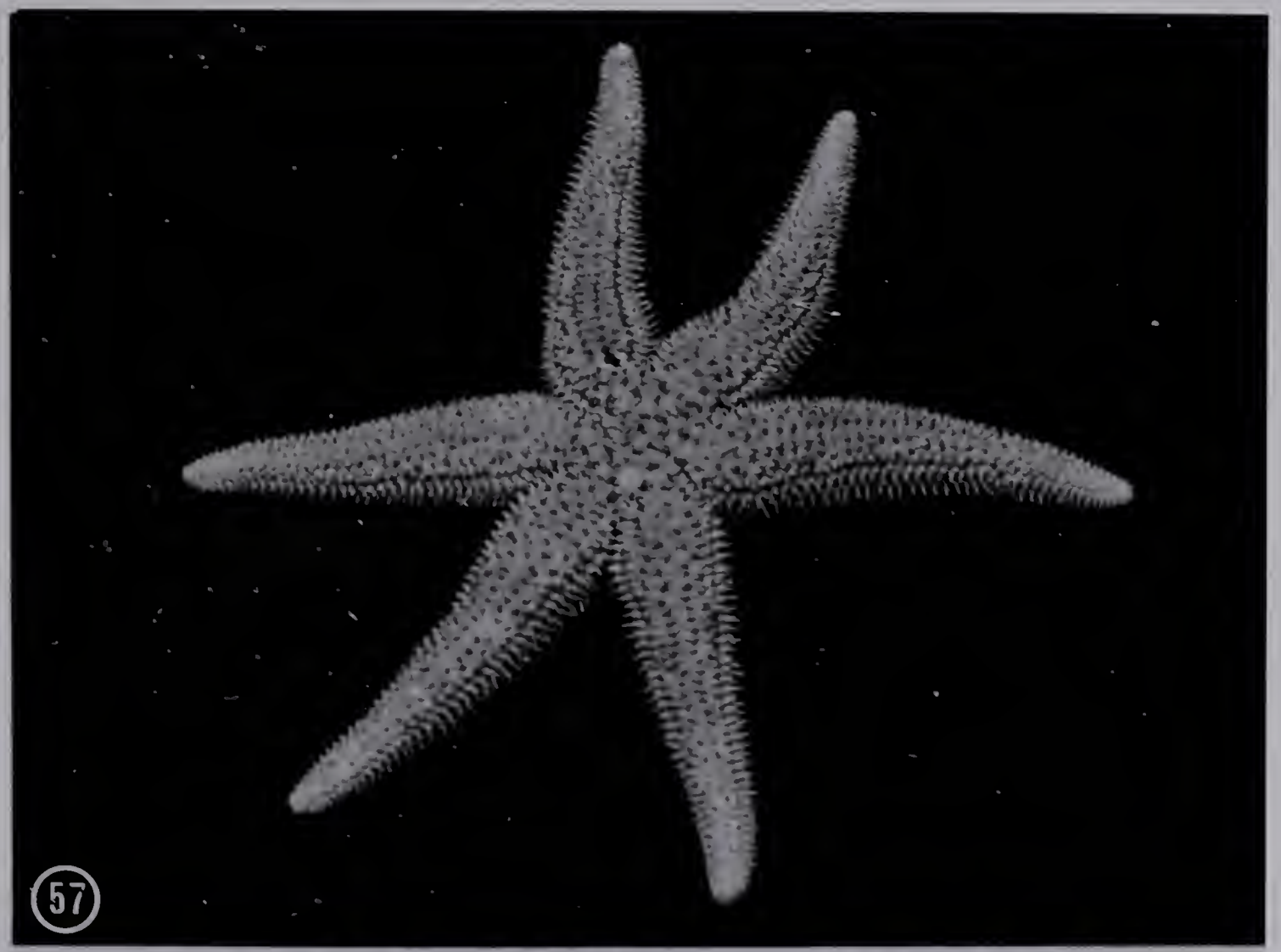
		Gland Type A	Gland Type B	Gland Type C	Gland Type D
length		56 μ	56 μ	56 μ	56 μ
width	base	9 μ	8.4 μ	7 μ	?
	neck	2.5 μ	2 μ	2 μ	?
secretory particle diameter		4.2 μ	0.9 - 0.5 μ	1.5 μ	0.7 μ
chemical composition of gland cell		acid muco-polysaccharide	no apparent reaction	possible protein	?
opening to exterior		yes, pore	yes, pore	yes, pore	?
approximate ratio in epidermis		80%	10%	10%	1%
location of nucleus		edge of base	base	base	?
distribution in epidermis		entire	entire	entire	?
Remarks			secretory packets in upper 14 μ of cell only	secretory packets in upper 18 μ of cell only	

Fig. 57. Aboral surface of Leptasterias hexactis. Dried specimen. X½.

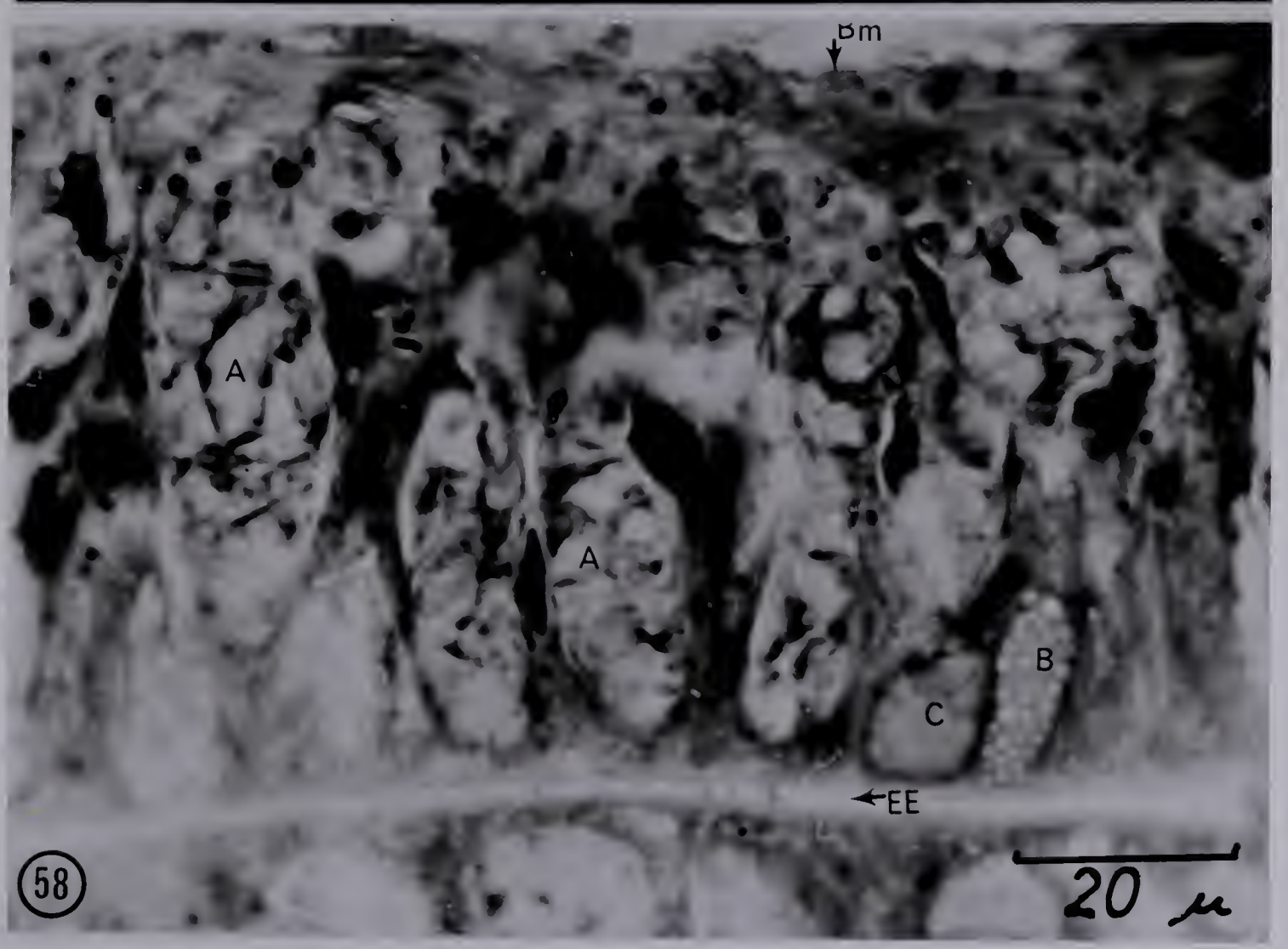
Fig. 58. Cross section through the aboral epidermis of Leptasterias hexactis.

A, Type A gland cells; B, Type B gland cells; Bm, basement membrane; C, Type C gland cells; EE, external environment.

Bicarbonate/osmium fixation, Epon 812 embedding, and Richardson's staining.



57

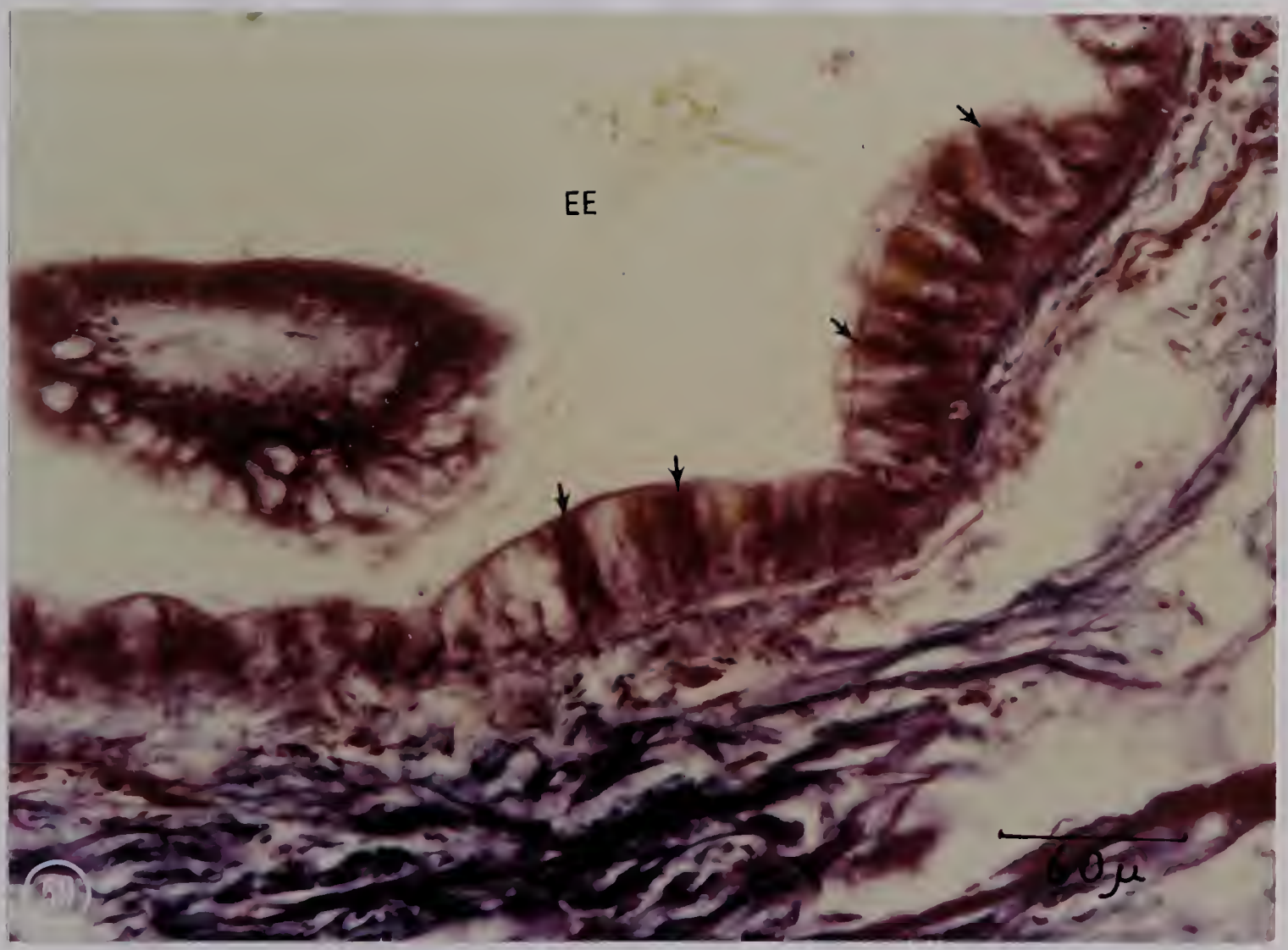


58

Fig. 59. Cross section through the aboral surface of *Leptasterias hexactis* exhibiting Orange G positive gland cells.

EE, external environment; arrows indicate the position of Orange G positive gland cells.

SUSA fixation, Paraplast embedding, and One Step Mallory staining.


EE
60μ

IV. DISCUSSION

1. Previous Theory: Mucus and Muriform Glands.

The Phylum Echinodermata has been long neglected by histologists in spite of the ready availability of many species. The main reason for this is that the calcium endoskeleton has represented a distinct barrier to histologists who found it easier to section noncalcified animals. This, together with the fact that they have little economic importance, has slowed histological research in this field since the late nineteenth century.

Cuénot in 1887 suggested that there were two basic types of gland cell common to all asteroids, but which varied in abundance with region and species. The two gland cell types were:

1. The mucus gland cell which was reported to contain finely granular contents.
2. The muriform gland cells, which were reported to contain coarse particles.

To date, most of the structures thought to be glandular have been placed in either of these categories. The advent of plastic embedding media have enable more precise structural observation than had previously been possible. This new technique has enabled me to show that in eight species of

asteroids all of the gland cells contained distinct secretory vesicles with no evidence of the coarse particles or fine granular contents as suggested by Cuénot. In contrast, by using wax embedding I have produced the structures described by Cuénot (Fig. 40).

In my opinion the reason for this apparent discrepancy is directly related to the initial fixation and embedding of the tissue in question. All previous work in studying these cells had been done with tissue embedded in compounds other than synthetic resins. These techniques tend to produce artifacts in the tissue being treated. When fixed and embedded in wax the gland cells on the aboral surface of the asteroids studied appeared to react in one of two ways. Either the secretory packets burst and their contents formed a uniform "mucus" precipitate bounded by the cell wall of the gland, or the secretory packets became changed to form what appeared to be coarse "muriform" particles. The reason for this lies in the paraffin technique which should not be used for structural identification in this tissue. On the other hand sections cut from tissue embedded in Epon 812 showed little distortion due to fixation or embedding. Studies of sections from tissue embedded in Epon 812 clearly show that the difference between the mucus and muriform gland cells is an artificial distinction stemming from attempts to interpret physically distorted tissue produced by the paraffin embedding method. Paraffin embedded

tissue should be abandoned as a basis for classifying and for studying glandular structure.

2. Epidermal structure

In many asteroids the epidermal structure is distinctive. Thus, in the genera examined, the histological picture in each is peculiar to the genius and the source of the material can be recognized at a glance from the slide.

A. Cuticle

One common structure which is shared by all the asteroids studied (with the possible exception of Luidia foliata) is the cuticle which covers the epidermis. When this structure is viewed with the light microscope it appears to be a continuous solid covering which has varying degrees of affinity for Richardson's stain which produces the appearance of layering in the structure. The cuticle is pierced only by a pore which occurs at the tip of some mature gland cells, and by the cellular projections which will be referred to later. The asteroid cuticle was described by J. E. Smith in 1937, who suggested that it consisted of two layers. This does not appear to be the case in the animals which I have studied, as no apparent layering was observed in some species and up to three layers in others. However, not enough animals have been examined to be able to suggest whether or not this

is a species specific or a variable phenomenon.

When the cuticle of Dermasterias imbricata and Leptasterias hexactis has been viewed with the electron microscope, cellular projections are shown to exist in the cuticle which cannot be clearly defined with the light microscope (Appendix III). I would tentatively expect these structures to be present in other cuticle-bearing asteroids. The cellular projections contained in the cuticle run from the cytoplasm of the epidermal cells to the surface of the cuticle but do not extend beyond it. Where the tubules reach the surface of the cuticle a distinct electron dense structure occurs. Some evidence of secretion from the cellular projections to the environment exists (Appendix III), but as the tip structure has not been sufficiently examined, this cannot be verified. In most cases, the cellular projections will go directly to the cytoplasm of the epidermal cell, but at other times two or more of them appear to join to form a single tube near the surface, suggesting a complex network.

In the cuticle of Polymorphus minutus (Acanthocephala), Crompton and Lee in 1965 described cuticular pores which were suggested to aid in the obtaining of nutrients through the otherwise impervious cuticle. In the asteroids this function would be unlikely in view of their well developed and highly functional stomachs. With the initial indication of secretion by the asteroid cytoplasmic projections as observed in Dermasterias imbricata (Appendix III) and Leptasterias hexactis it is conceivable that they could be involved in ionic

regulation or a passage to the exterior for the release of certain waste substances. The latter theory may be supported by Nicol (1960) who states in his book. The Biology of Marine Animals , that in the echinoderms part of the amino-acid nitrogen compounds which form the excretory products are not discharged via the excretory organs proper, but as leakage across the body surface. As it appears that the cuticle would be impervous to such substances, they would have to be released by either a glandular discharge or through the cellular projections which appear to pierce the cuticle. If these compounds were caught and retained for a time in the mucus on the surface of the animal, the possibility occurs that they could be of a toxic or irritating nature to other animals, and as such would augment or perhaps be the defensive component of the mucus.

B. Distinctive Epidermal Features

As previously stated, many of the asteroids have distinctive features in regard to their epidermal region, in addition to cuticular and glandular components.

Hippasterias spinosa has an epidermis in which long columnar cells containing large quantities of small, cytoplasmic vacuoles in the upper half of the cytoplasm occur. (Figs. 24, 25). This is the only animal which has a large number of vacuoles in the cytoplasm.

Both Henricia leviuscula and Luidia foliolata show a distinct difference between the epidermal area and the underlying tissue, but no distinct cell walls are present when viewed with the light microscope. The use of the electron microscope would be helpful in clarifying this problem.

The epidermal regions of both Pycnopodia helianthoides (Fig.52) and Pteraster tesselatus (Fig.39) appear to be composed entirely of glandular tissue. This too, could be clarified by the use of the electron microscope.

Dermasterias imbricata has no distinct basement membrane visible to light microscope (Fig.6). The tissue surrounding the gland cells in the epidermal region appear to be continuous with the tissue found below it. Furthermore, no distinct cells can be observed in this layer with the light microscope. The electron microscope shows however that distinct cells are present in this region. It is possible that this animal has an extreme modification of the dermal-epidermal structure in conjunction with the lack of a reinforcing calcium endoskeleton in this area, or perhaps the basement membrane exists either in a modified form or as a very thin structure which follows intimately the outline of the gland cells.

The epidermis of Mediaster aequalis occurs only in the primary and secondary interpaxillary grooves and in Henricia leviuscula (Fig.45) between the calcium spines and

in some of the grooves of the spines. In both cases the epidermis appears to be composed to a large extent of glandular tissue with some columnar cells present. It is possible that the entire epidermis is glandular and the columnar cells are juvenile gland cells. Where calcium spicules pierce the epidermis, they appear to be covered by the cuticle, possibly secreted by the tissue components which occur between the deposits of solid calcium.

Leptasterias hexactis gives the impression of having distinct columnar cells among the glandular tissue, but again they could be juvenile gland cells. Because no work has been done on the cycling of the gland cells, it is impossible to state if, in fact, these columnar cells are a separate tissue from the gland cells.

It has been generally accepted that one characteristic of the Class Asteroidea was a flagellated epidermis (Hyman 1955, Villee et al 1963, Barnes 1963). In this work no cilia or flagella were encountered on the aboral surface of Dermasterias imbricata, Pycnopodia helianthoides, or Pteraster tesselatus. The remainder of the asteroids had cilia or flagella in varying degrees depending on the species. I feel that it is a general misconception to state that the entire class has a ciliated or flagellated epidermis.

3. General Gland Structures

Asteroid aboral gland cells should not be classified by their structure entirely. Any classification should take into account the chemical nature of the products which are secreted. Two gland cells may appear physically identical, but secrete entirely different compounds (Mediaster aequalis, gland types B & C), and so this fact must be taken into account in any new form of classification.

There are two entirely structural criteria which could be used in a system of gland classification.

A. Gland Position

(a) Superficial gland cells in which the entire cell occurs only in the epidermal region of the animal, above the basement membrane when it is present. This gland grouping is typified by the gland cells found in Pycnopodia helianthoides.

(b) Deep gland cells, which have their necks only in the epidermal region and have the greater part of their bodies below the epidermal layer. The body of the cell usually occurs between the calcium spicules of the endoskeleton. Examples, of this type of gland are the Type C gland cells in Hippasterias spinosa.

B. Secretory Packet Release

(a) In the first case a distinct pore through the cuticle is present which exposes the secretory packets to the environment. This condition is shown by the Type A gland cells of Pycnopodia helianthoides.

(b) In the second case, no distinct pore is observed, but the surface of the cell wall appears to rupture liberating the secretory packets. This is exhibited by the Type D gland cells of Pteraster tesselatus.

There are other structural criteria which might prove useful in a classification method for asteroid aboral gland cells, but not enough is known to apply them as yet.

All asteroid gland cells observed to date exhibit the common characteristic of containing secretory packets. These are membrane bounded structures which carry the products of the cell to the environment. In many cases the secretory packets appear to fill the cell body to the exclusion of all other structures. The assumption is that these are mature gland cells ready to discharge their contents to the exterior.

Stages of the gland growth have been observed in very few cases, and only one case, the Type C gland cell of H. spinosa, does it appear that continuous production of secretory packets from the base of the gland cell occurs.

There appear to be three possible explanations for this lack of juvenile gland cells:

1. The gland cells may have a periodic cycling where at certain times the gland complex is discharged and rebuilt.

2. The gland cell may be more or less dormant until it is required to discharge its contents after which the cell may be rebuilt.

3. The immature gland cells may not have been seen in the animals worked on or may have not been recognized as such.

In any event, more basic studies in the recycling of gland cells are required in order to be able to separate the gland types so that two gland types will not be described where in fact two stages of the same gland occur.

In other cases, the secretory packets occur only in the top half of the gland cell with the nucleus and cytoplasm below them and no opening to the exterior. This type of cell may be an immature form of one of the recognized mature cells, but this appears unlikely due to its lack of any demonstrable chemical substance.

4. Specific Gland Structure

One basic form of gland exists in all asteroids. This common type is vase-shaped and may or may not have an opening to the exterior. The secretory packets can either occur in the entire cell, or be confined to the top part of the cell. The nucleus appears in the bottom half of the cell. The secretory packets in this gland cell contain an amorphous material. Cytoplasm may or may not be conspicuous in the cell.

In conjunction with the above gland form variations in different animals are observed.

In Dermasterias imbricata a massive, apparently glandular complex occurs about 600 μ. from the surface of the animal and is separated from the epidermal region by connective tissue and collagen. It is composed of a bead-like chain of single, spherical cells. This column of cells is separated from neighboring columns by connective tissue and collegen fibres. The cells which form the columns are filled with distinct secretory packets around a central nucleus. An apparent progression of development was observed from the bottom to the top of the column, but no means for the release of these cells had been found. Their function is unknown.

Luidia foliolata has as its major gland cell a structure of uniform diameter and of great length filled with secretory

packets. It appears as a tube doubled back on and twisted around itself in the lower parts of the epidermis. There is no way of knowing, at this point, if it is composed of one tube, or if it is a series of branched tubes. At certain intervals it sends a tube to the surface to a distinct secretory pore in the cuticle.

In Hippasteria spinosa, the Type B gland cells produce what appear to be very thick walled, spherical secretory packets which are liberated by a cuticular pore. The Type C gland cells which are of extreme length and having secretory packets in the top two-thirds of the cell have irregular cytoplasmic contents below this in place of normal secretory packets.

Pteraster tesselatus has Type C gland cells which have a dense sphere in the centre of clear secretory packets and Type E gland cells which have a dense compound enclosed in their secretory packets.

Henricia leviuscula has an unusual glandular packet which has two nucleated glandular cells surrounded by an apparent nucleated matrix of secretory packet of either a third cell type or a syncytium.

Pycnopodia helianthoides contains Type C gland cells in which the interior does not contain secretory packets but rather a material which appears in the form of a continuous thread.

The signifigance of these structural variations from the more common gland cell type is not fully understood. In some cases it appears to be due to the product which they secrete. For example, the Type B gland cells in Pteraster tesselatus which have a distinct secretory packet, apparently lipid material, with a dense proteinaceous sphere in their centre. In other cases it may be linked to the ecology of the animal. An example of this may be the secretory packet which occurs in Henricia sanguinolenta which possibly aids in supplying mucus for its suggested filter feeding. Further work is needed to understand how and why these gland types are necessary to the animal.

5. Gland Histochemistry

The chemical tests, as far as can be determined to this date, indicate two major gland types, with other types occurring less frequently.

The first major chemical component that is found in gland cells is acid mucopolysaccharide. It occurs in certain gland cells in all of the asteroids in this study. However, the tests for this compound suggest that it was not all of the same type, and if acid mucopolysaccharide from different asteroids was subdivided into its different types, these types could very well not be identical for each animal. This speculation is based on the observation that different asteroid gland cells containing acid mucopolysaccharide showed slight

differences in their M.B.E. (Methylene Blue Extinction) points, alcohol labile as contrasted to alcohol fast metachromasia was observed, and staining times for acid mucopolysaccharide containing gland cells were varied. For these reasons I feel additional investigation into this area might prove profitable.

The second major chemical component found in certain gland cells was that substance which showed a positive P.A.S. reaction. Gland cells showing a positive response to the test occurred in all the animals used with the exception of Luidia foliolata. Leptasterias hexactis produced a marginal response which may or may not indicate P.A.S. positive material, but all other asteroids gave definite responses in certain gland cells to this test. The response in most cases was probably due to neutral mucopolysaccharide, but the P.A.S. test will show other compounds such as muco- and glycoproteins, glycolipids, and phosopholipids. It is necessary for further histochemical work to be done to separate the reacting substance as precisely as possible. In addition to individual gland cells giving a response for either acid mucopolysaccharide or for P.A.S. positive compound, both of these substances may be secreted from the same gland cell. In this case, that cell would show as positive for both the acid mucopolysaccharide tests and for the P.A.S. reaction. It can be difficult to tell the three types of cells apart, especially where they appear to be intermingled as in Pycnopodia helianthoides.

In addition to these two substances which comprise the majority of the chemical components in the asteroid gland cells, I observed two other chemical compounds occurring in some of the asteroid gland cells. These types of cells are not as common as the previous types, but may be very important.

The first type are those gland cells which stain with Fast Blue B in conjunction with the D.D.D. reaction indicating the presence of protein. This type of gland cell occurs in L. hexactus, M. aequalis, D. imbricata, and P. tesselatus. In M. aequalis, the reacting cell appears to be the same as the gland cell which gives a light, but positive P.A.S. response. This suggests the presence of a mucoprotein. In the other three animals no such correlation is indicated. One fact which has no histochemical significance, occurs in all four of the above animals however. In these animals the same gland cells that react positively to the D.D.D. test also will stain brilliantly with orange G. The orange G. also stains the muscle of the asteroid and especially the muscle which closes the pedicellariae on those animals which possess them. Other histochemical reactions for protein should be tried to see if a verification for the positive result can be obtained and then to see if a specific type of protein can be established.

Why should a starfish secrete protein? In my opinion two possible reasons for this type of secretion would be to the advantage of the animal.

1. This type of gland may secrete a toxin or irritant which would use the mucus as a carrying vehicle and perhaps to prevent its being dispersed too rapidly and thus losing its effectiveness through dilution with ocean water.

2. It may be an enzyme producing gland cell to digest the mucus after it has been released in quantity in response to irritation, allowing the water to come into close proximity to the dermal branchiae for respiration.

Should the mucus remain on the surface of the animal in any quantity and interfere with the dermal branchae, the respiratory ability of the tube feet might not be sufficient and death may ensue. This is especially true in the case of Pteraster tesselatus which has its dermal branchiae in an enclosed area in the nidimental chamber (Fig.32). It is interesting to note that the floor of the nidimental chamber, the true epidermis, has the highest concentration of cells which are D.D.D. positive and apparently secreting protein. In view of this enclosed respiratory system and the ability to produce large amounts of mucus, these non-carbohydrate containing cells could well be producing a compound to remove the mucus from the surface of the animal.

The second type of cells were found in all eight species of asteroids studied, but not in the same physical

form. In this case they are cells that can be observed only in sections cut from Epon 812 embedded tissue. They appear to have no affinity for any of the histochemical tests tried or any of the other stains used. They appear to be further unique in that they do not appear to have an opening to the exterior of the animal. In Pycnopodia helianthoides the Type A gland cell falls into this category (Fig.53). These cells structurally resemble what one would expect to see in a juvenile gland cell of Type B (Fig.52), except for the fact that a juvenile gland cell at this stage of development would be expected to give chemical reaction of some kind even if it were not the same as the mature cell. As their glandular contents, (ie. the secretory packets) occur only in the upper half of the cell, they are easily distinguished from other gland cells in P. helianthoides because the remaining gland cells have secretory packets filling the entire cell, and any stain will run from the basement membrance to the surface. If these glands do not have an opening to the exterior, and they are not a juvenile gland form, then their functioning can be determined only by further research. As their chemical components appear to be neither an acid mucopolysaccharide nor a P.A.S. positive material, and do not appear to contain protein or give a lipid reaction, it must be assumed that they contain some other substance. This is with the reservation that the lipid analysis is questionable as will be seen later. In the Epon 812 embedded section, these gland cells appear to contain perfectly

clear secretory packets, with no trace of any opaque material as contained in the other gland cells. With this in mind, it is possible that they might contain water, possibly with perhaps a high ionic content which would not be shown with the histochemical techniques that were used.

The possibility that gland cells containing lipid material are present can be suggested, but not confirmed at this time. This is due to the fact that the apparatus for the extraction of lipid material was not available to verify the results of the lipid soluble dyes used. Without this confirmation, the results of the histochemical tests are only indicative of the presence of lipid material. To compound this problem, the epidermis of most asteroids appeared to contain a high fraction of lipid material. This view is based on the strong positive response given by the epidermis when tested for lipid. The response tends to block out all cellular detail to such a degree that it is not known whether or not the gland cells in the area are positive. The reason for this apparent positive lipid reaction may be due to the pigments which give the animals their coloration. Carotenoids are common in the asteroid integument (Nicol, 1960). Carotenoids are soluble in lipids and in typical lipoid solvents, but insoluble in water. When all of the asteroids in this study were examined microscopicly in unfixed sections, the epidermal region was observed to contain diffuse colors throughout its entire area, which

varied with species. As carotenoids are soluble in lipid and as there are diffuse colors not confined to specific cells in the asteroids studied, it is possible that a lipid in the epidermis could be responsible for the diffuse coloration in this area.

In order to see if specific glandular tissue is responding to the lipid soluble dyes, very thin cryostat sections would need to be cut and submitted to lipid histochemical tests. This may allow the individual parts of the tissue to respond to the lipid soluble coloring agent in a more selective and reduced manner than has been possible in the time available for this study. However, in spite of these difficulties, there is a possibility of gland cells containing lipid material in an unknown gland cell in the epidermis of Hippasterias spinosa and in the Type C gland cells of the same animal.

The most difficult problem in regard to the histochemical tests is to correlate the histochemical reaction observed in Paraplast or gelatin embedded reaction to similar tissue embedded in Epon 812. This difficulty could be solved in part by the use of the P.A.S. - Alcian Blue reaction and the Hale colloidal iron method on plastic embedded tissue (Ito, 1965). Water soluble Resin embedding may also prove useful in histochemical tests. (Gibbons 1960, Staubli 1963, Leduc, 1965).

V. CONCLUSIONS

The gland cells which occur in the aboral surface of asteroids appear to be far more complex than previously suspected. Hyman states in volume 4 of The Invertebrates that, "The secretion of mucus must be regarded as playing an important role in the biology of asteroids." Yet very little is known about either the mucus or the cells which produce it.

It is not known how the mucus is used by the animal in relation to its environment. Based on this study, in my opinion, it would not be wise to read too much into the various responses produced in other animals by asteroids until the gland cells can be accurately identified and classified according to their chemical components. In this way, it may be possible to ultimately determine if the specific substances responsible for the reactions caused in other animals by asteroids are produced by the epidermal glands. It may be that products released by the epidermal cells and not the mucus per se are responsible for these reactions. The appearance of cellular projections in the cuticle which may have a secretory function, and references to coelomocyte excretion (Nichols, 1962) through the epithelium, suggests the need to study the epidermal region as a whole, and not only as isolated glandular tissue, in order to understand the total effects of the secreted products.

Far more work is needed in this long neglected field in order to attempt the understanding of the basic interaction of asteroids with their environments.

VI. LITERATURE CITED

Baker, J. R. 1963. Cytological Technique. Methuen, London. 4th Ed.

Barka, T., and P. J. Anderson. 1963. Histochemistry. Harper and Row, Inc. New York.

Barnes, R. D. 1963. Invertebrate Zoology. W. B. Saunders and Company. Philadelphia and London.

Carleton, H. M., and R. A. B. Drury. 1957. Histological Technique. Oxford University Press. London. 3rd Ed.

Cason, J. E. 1950. A rapid one-step Mallory-Heidenhain stain for connective tissue stain. Stain Technol. 25: 225.

Crompton, D. W. T., and D. L. Lee. 1965. The fine structure of the body wall of Polymorphus minutus (Goeze, 1782) (Acanthocephala). Parasitology, 55: 357-364.

Cuénot, L. 1887. Contribution a l'étude anatomique des asteroids. Arch. Zool. Exp. Gen., 5, 2me. memoire. Pp. 135.

Culling, C. F. A. 1963. Handbook of Histopathological Techniques. Butterworths. London. 2nd Ed.

Davenport, H. A. 1960. Histological and Histochemical Techniques. W. B. Saunders Co. Philadelphia.

Emmel, V. M., and E. V. Cowdry. 1964. Laboratory Technique in Biology and Medicine. Williams and Wilkins Company, Baltimore.

Feder, H. , and A. M. Christensen. 1966. Aspects of asteroid biology. p. 93 - 107. In R. A. Boolootian (ed.) Physiology of echinodermata. John Wiley and Sons, New York.

Fisher, W. K. 1911. Asteroidea of the north Pacific and adjacent waters. Smithsonian institution. United States National Museum. Bulletin 76.

Fisher, W. K. 1928. Asteroidea of the north Pacific and adjacent waters. Smithsonian institution. United States National Museum. Bulletin 76.

Fisher, W. K. 1930. Asteroidea of the north Pacific and adjacent waters. Smithsonian institution. United States National Museum. Bulletin 76.

Gibbons, I. R. (1960). Proc. 4th Intern. Congr. Electron Microscopy 2, 55. Springer, Berlin. Cited by Pease, D. C. Histological Techniques for Electron Microscope. 1964. Academic Press. London and New York. 2nd ed.

Hayashi, R. 1935. Studies on the morphology of Japanese sea - stars. J. Fac. Sci., Hokkaido Imp. Univ., series VI, zoology, vol IV no. I.

Hyman, L. H. 1955. The Invertebrates. IV. Echinodermata. McGraw Hill, New York.

Ito, S. 1965. The entire surface coat on cat intestinal microvilli. J. cell Biol. 27: 475-492.

Leduc, E. H. and S. J. Holt. 1965. Hydroxypopyl methacrylate, a new water miscible embedding medium for electron microscopy. J. Cell Biol. 26: 137 - 156.

Luft, J. H. 1961. Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. 7: 409 - 414.

Mortensen, T. and I. Lieberkind. 1928. Die Tierwelt der Nord- und Ostsee. Teil 8. Echinodermata.

Nichols, D., 1964. Echinoderms, Hutchinson University Library. London. 2nd Ed.

Nicol, J. A. C. 1960. The biology of marine animals. Sir Issac Pitman and Sons Ltd. London.

Pantin, C. F. A. 1962. Notes on microscopic technique for zoologists. Cambridge Univ. Press.

Pearse, A. G. 1961. Histochemistry, theoretical and applied. J. and A. Churchill Ltd., London. 2nd Ed.

Richardson, K. C., L. Jarett, and E. H. Finke. 1960. Embedding in epoxy resins for ultra thin sectioning in electron microscope. Stain Techol. 35: 313 - 325.

Richetts, E. F. and J. Calvin. 1956. Between Pacific Tides. Stanford University Press. Stanford, California. 3rd Ed.

Rio, G. J., G. D. Ruggieri, M. R. Stempien, Jr., and R. F. Nigrelli. 1963. Saponin-like toxin from the giant sunburst starfish, Pycnopodia helianthoides, from the Pacific northwest. Amer. Zool., 3: 554 - 555.

Rodenhouse, I. Z., and J. E. Guberlet. 1946. The morphology and behaviour of the cushion star Pteraster tesselatus Inves. Univ. Wash. Pub. Biol. Seattle. 12: 21 - 48.

Smith, J. E. 1937. On the nervous system of the starfish Marthasterias glacialis. Phil. Trans. Roy. Soc. Ser. B 227: 111 - 133.

Staubli, W. 1963. A new embedding technique for electron microscopy, combining a water soluble epoxy resin (Durcupan) with water insoluble Araldite. J. cell biol. 16: 197 - 201.

Sund, P. N., 1958. A study of the muscular anatomy and swimming behaviour of the sea anemone, Stomphia coccinea. Quart. J. micr. Sci. 99: 401 - 420.

Villee, C. A., W. F. Walker, and F. E. Smith, 1963. General Zoology. W. B. Saunders Company. Philadelphia and London.

Ward, J. A. 1965. An investigation on the swimming reaction of the sea anemone Stomphia coccinea. II. Histological location of a reacting substance in the asteroid Dermasterias imbricata. J. exp. Zool. 158: 365 - 372.

Watson, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. J. biophys. biochem. Cytol. 4: 475 - 478.

Yentsch, C. S., and D. C. Pierce, 1955. "Swimming" anemone from Puget Sound. Science. 122 (3182) 1231 - 1233.

APPENDIX I

Tables showing the results of the histochemical tests used on the aboral asteroid gland cells which compared to positive results from tissue other than asteroid.

lll - strong positive reaction

ll - medium positive reaction

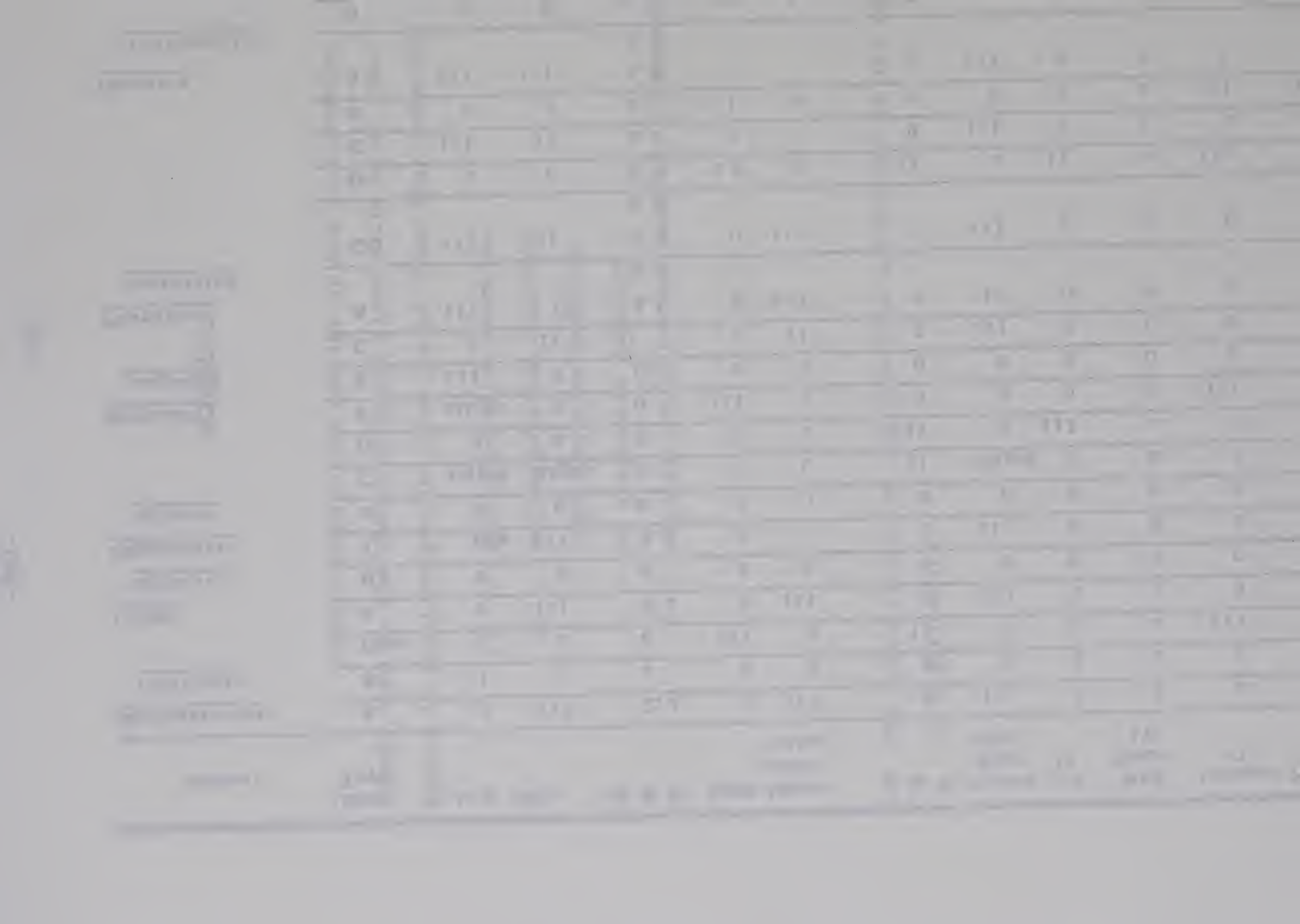
l - light positive reaction

0 - no observable reaction

? - reaction could not be observed, if present.

* The effectiveness of the Nile Blue sulfate due to its age is questionable.

Animal	Gland Type	P.A.S.	Hale	M.B.E.	DDD	Metachromasia	S.B.B.	Alcian Blue 8GX	Oil O	Red Sudan IV	Orange G	Ninhydrin
Dermasterias	A	0	111	2.2	0	111	0	111	0	0	0	0
imbricata	B	11	0	0	0	0	0	0	0	0	0	0
	Der.	0	0	0	111	0	11	0	0	0	111	11
Luidia	A	0	111	2.2	0	111	0	111	0	0	0	0
foliolata	B	0	0	0	0	0	0	0	0	0	0	0
Hippasteria	A	few	111	3.5	0	1	0	111	0	0	0	0
spinosa	B	0	0	0	0	0	0	0	0	0	0	0
	C	slight	slight	0	0	0	11	slight	0	0	0	0
	D	0	0	0	0	0	111	0	111	--	0	0
Mediaster	A	slight	0	0	111	0	0	0	0	0	111	?
aequalis	B	111	0	0	0	0	0	0	0	0	0	0
	C	0	11		0	11	0	111	0	0	0	0
Pteraster	A	111	11	3.5	0	111	0	111	0	0	0	0
tesselatus				2.2								
	GU	111	11	3.5	0	111	0	111	0	0	0	0
				2.2								
	B	0	0	0	11	0	11	0	11	--	111	?
	C	111	11	2.2	0		0	111	0	0	0	0
	D	0	0	0	111	0	0	0	0	0	111	11
Henricia	A	111	111	3.5	0		0	111	0	0	0	0
leviuscula				2.2								
	B	0	0	0	0	0	0	0	0	0	0	0



APPENDIX I (Continued)

Animal	Gland Type	P.A.S.	Hale	M.B.E.	DDD	Metachromasia	S.B.B.	Alcian Blue 8GX	Oil Red O	Red Sudan IV	Orange G	Ninhydrin
	G_1 G_2 G_3	NO TESTS DONE										
Pycnopodia helianthoides	A	0	0	0	0	0	0	0	0	0	0	0
	B	111	111	3.5 3.2	0	11	0	111	0	0	0	0
	C	--	--	--	--	--	--	--	--	0	--	--
	D	--	--	--	--	--	--	--	--	0	--	--
Leptasterias hexactus	A	0	111	3.5 2.2	0	111	0	111	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	111	0	0	0	0	0	111	1

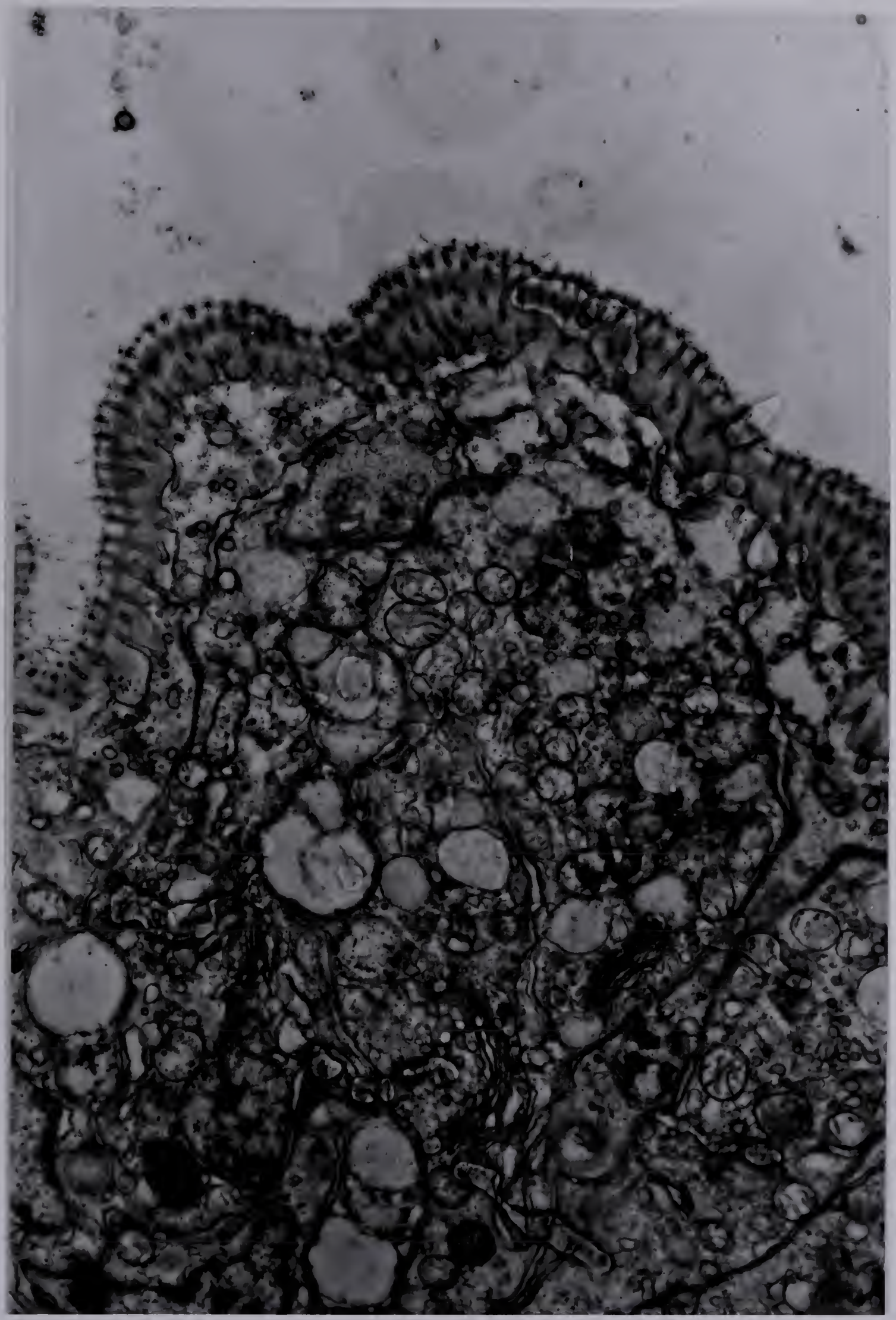
APPENDIX I (Continued)

Animal	Gland type	3:4 Benz-pyrene	Acri-dine orange	Phos-phene GN	Nile Blue sulfate *	Acetone Sudan black B	Burnt Sudan black B
Dermasterias	A	0	111	?	0	--	--
imbricata	B	0	0	?	0	--	--
	C	0	0	0	0	--	--
Luidia	A	0	111	0	0	--	--
foliolata	B	0	0	0	0	--	--
Hippasteria	A	0	111	0	0	0	0
spinosa	B	0	0	0	0	0	0
	C	0	0	0	0	1	11
	D	?	0	?	0	0	0
Mediaster	A	0	0	0	0	--	--
aequalis	B	0	0	0	0	--	--
	C	0	111	0	0	--	--
Pteraster	A	?	111	?	0	?	?
tesselatus	GU	0	111	0	0	0	0
	B	11	0	11	0	?	11
	C	0	111	0	0	0	0
	D	0	0	0	0	0	0
Henricia	A	?	111	?	0	0	0
leviuscula	B	?	0	?	0	0	0
	G_1 G_2 G_3	NO TEST DONE					
Pycnopodia	A	?	0	?	0	--	--
helianthoides	B	0	111	0	0	--	--
	C	--	--	--	--	--	--
	D	--	--	--	--	--	--
Leptasterias	A	?	111	?	0	--	--
hexactus	B	?	0	?	0	--	--
	C	?	0	?	0	--	--

APPENDIX II

The typical aboral epidermal structure of *Dermasterias* when viewed with the electron microscope.

Bicarbonate/osmium fixation, Epon 812 embedding, and uranyl acetate staining. 7,000 X.



APPENDIX III

An electron micrograph showing the details of the cuticle found on the aboral surface of Dermasterias imbricata. The canals through the cuticle appear prominently and show some evidence of secretion. Notice the apparent intimate contact of the epidermal cell cytoplasm with the base of the cuticular tubules, and also the suggestion of a possible connection of these tubules with the junction between the cells.

Bicarbonate/osmium fixation, Epon 812 embedding, uranyl acetate staining. X 10,000.